ON THE ANATOMY OF DESCENDING PATHWAYS FROM THE BRAIN STEM TO THE SPINAL CORD IN A LIZARD, VARANUS EXANTHEMATICUS

by
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Front cover: Dense serotin immunoreactive fiber plexus in the nucleus motorius nervi trigemini pars ventralis Thin varicose fibers and varicosities of various sizes, ranging from very fine to thick, surround motoneurons, strongly suggesting a terminal field

Back cover: Dense serotonergic fiber plexus in the rostral part of the nucleus descendens nervi trigemini Note that very fine to medium-sized ter minal structures are found in the dorsolateral part (left), whereas mainly thick and very thick varicosities are located in the ven trorostral part of this nucleus (right)

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ON THE ANATOMY OF DESCENDING PATHWAYS FROM THE BRAIN STEM TO THE SPINAL CORD IN A LIZARD, VARANUS EXANTHEMATICUS

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ter verkrijging van de graad van doctor in de geneeskunde aan de katholieke universiteit van Nijmegen, op gezag van de rector magnificus prof. dr. J.H.G.I. Giesbers, volgens besluit van het college van dekanen in het openbaar te verdedigen op

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PROMOTOR: PROF. DR. R. NIEUWENHUYS
CO-REFERENT: DR. H.J. TEN DONKELAAR

Overziet men tenslotte het geheel, dat ik U heden voorlegde, dan blijkt, dat de emotie een reactie is van het geheele individu ten aanzien van zijn omgeving. Geplaatst tegenover een wereld van verlokking of bedreiging, welke zich aan mensch of dier opdringt door de poorten van zijn zinnen, wordt het individu genoopt zijn houding ten aanzien van deze wereld te bepalen, een houding van toewending of afkeer. Deze positiename bepaalt het correspondeerende uitwendige gedrag, hetwelk de verhouding individu-milieu kan wijzigen.

Aan dit gecompliceerde psychologische spel ligt als materieel substraat ten grondslag een machtig centraal apparaat, dat door middel van zintuig- en periphere zenuwen eenerzijds met de buitenwereld en alle orgaansystemen van het eigen lichaam verbonden is, terwijl het anderzijds zijn invloed kan doen gelden op alle lichaamsorganen dank zij de aanwezigheid van efferente zenuwverbindingen.

Op deze wijze bezien wordt het duidelijk, hoe in het emotioncel gebeuren het geheele individu mee resoneert, hoe zich hierbij een prachtig harmonisch spel ontplooit van psychische- en somatische processen, welke elkaar wederzijds beïnvloeden en hoe wellicht nergens duidelijker dan in de emotie tot uitdrukking komt de twee-eenheid der menschelijke en dierlijke natuur.

Prof. Dr. J.J.G. Prick (1945) in: "Het Gevoelsleven", inaugurale rede, uitgesproken bij het aanvaarden van het ambt van buitengewoon hoogleraar in de psychologie aan de Katholieke Universiteit te Nijmegen.

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Recent studies (see e.g. Kuypers and Maisky 1975, 1977; Peterson et al. 1975, 1978, 1979; Basbaum et al. 1978; Holstege et al. 1979; Tohyama et al. 1979a,b; Holstege and Kuypers 1982; Martin et al. 1975, 1979a,b,c, 1981, 1982; Hayes and Rustioni 1981; Bowker et al. 1981, 1982; Björklund and Skagerberg 1982) with modern tracer, immunohistochemical, and physiological techniques in various mammals have shown that the organization of descending supraspinal pathways is much more complex than previously known. Moreover, such studies revealed a number of new pathways, arising in the hypothalamus, the nucleus Edinger-Westphal, locus coeruleus, dorsal column nuclei, which were not demonstrated with the classical anterograde and retrograde degeneration techniques (reviewed by Walberg, 1982).

More in particular these modern tracer studies have enlarged and refined our knowledge about the reticulospinal pathways. Focussed projections were demonstrated from certain reticular regions to e.g. the spinal laminae I and II (superficial layers of the dorsal horn), to the lateral part of laminae IV to VII, to laminae VII and VIII, to lamina X, to the intermediolateral cell column, and to the sacral parasympathetic nuclei (Martin et al. 1979b,c, 1982; Loewy et al. 1979; Holstege et al. 1979; Holstege and Kuypers 1982).

In addition, direct projections from particular reticular areas to spinal motoneurons were established with anatomical (Martin et al. 1979b,c, Holstege et al. 1979; Holstege and Kuypers 1982) and physiological (Grillner and Lund 1968; Wilson and Yoshida 1969; Wilson et al. 1970; Shapovalov 1975; Peterson et al. 1978, 1979; Peterson 1979, 1980) techniques. Excitatory and inhibitory reticulospinal neurons are differentially localized in the medullary reticular formation et al. 1970; Peterson et al. 1978). The same holds for reticulospinal neurons that project to limb, axial, and neck motoneurons, respectively. The wide variety of input sources of the reticular formation (see Peterson 1979, 1980 for reviews), in combination with its extensive and differentiated spinal projections, suggests a major role of this central brain stem structure in the control of posture, the regulation of axial and proximal limb musculature, and in the generation of alerting and orienting responses (Peterson and Fukushima 1982).

Specific brain stem nuclei as well as cell clusters which cannot easily be delineated in normal material, have been shown, with histofluorescence and immunohistochemical techniques, to contain large amounts of catecholaminergic, serotonergic, or peptidergic neurons (Dahlström and Fuxe 1964; Nobin and Björklund 1973; Hökfelt et al. 1977a,b; Cuello and Kanazawa 1981; Finley et al. 1981; Steinbusch 1981; Sladek et al. 1982). Projections from many of these cell groups to the spinal cord have been demonstrated (Nygren and Olsson 1977; Satoh et al. 1977; Basbaum and Fields 1979; Loewy et al. 1979a,b; Westlund and Coulter 1980; Westlund et al. 1981, 1982; Björklund and Skagerberg 1982; Martin et al. 1982). The aminergic and presumably also peptidergic reticulospinal components modulate the locomotion pattern generated at the spinal level (Grillner 1975; Wetzel and Stuart 1976; Kuypers 1982).

The descending pathways to the spinal cord arising from the raphe nuclei, which are serotonergic, and partly peptidergic by nature (see Hökfelt et al. 1979; 1980; Bowker et al. 1981, 1982, 1983), terminate in all parts of the spinal grey matter. The raphe projections to the dorsal horn, laminae I and II, have been shown to play an important role in pain modulation (Basbaum and Fields 1978, 1984; Abols and Basbaum 1981; Beitz 1982a,b,c; Gobel et al. 1982; Miletic et al. 1984; Dubner and Bennett 1983).

The phenomenon of axon collateralization was first demonstrated with the Golgi technique by Cajal (1909). Electrophysiological studies, focussed on the red nucleus (Shinoda et al. 1977), the vestibular nuclear complex (Abzug et al. 1973, 1974), and the reticular formation (Peterson et al. 1975, 1979), gathered evidence for the existence of considerable amounts of neurons in the brain stem, that give rise to branching axons which project to widely separate levels of the spinal cord. Recently developed multiple retrograde tracer techniques (Hayes and Rustioni 1979; Olsson and Kristensson 1978; Kuypers et al. 1980) supplied further evidence for the existence of extensive collateralizing pathways from various brain stem nuclei to the spinal cord (Hayes and Rustioni 1981; Huisman et al. 1981, 1982).

A somatotopic organization of several descending supraspinal pathways in mammals was demonstrated in classical degeneration studies as well as with electrophysiological and modern tracer techniques. Such somatotopic arrangement was found e.g. in the rubrospinal (Pompeiano and Brodal 1957; Miller and Strominger 1973; Shinoda

et al 1977, Hayes and Rustioni 1981, Huisman et al 1981, 1982), the vestibulospinal (Brodal et al 1962, Abzug et al 1973, 1974, Hayes and Rustioni 1981), and reticulospinal (Peterson et al 1975, Zemlan and Pfaff 1979, Satoh 1979) pathways

The descending pathways from the brain stem to the spinal cord can be divided into a medial and a lateral group as advocated by Kuypers (1964, 1982) The medial component, descending to the spinal cord via the ventral funiculus, comprises interstitiospinal, reticulospinal, and vestibulospinal pathways, and mainly terminates in the ventral part of the intermediate zone, supplying information for trunk and proximal limb musculature The lateral system, descending via the lateral funiculus, and comprising the contralateral rubrospinal and pontospinal pathways, mainly terminates in the dorsal part of the intermediate zone, supplying information for distal limb muscles In addition, a third component of the motor system has been postulated, based on recent findings with several modern techniques (see Kuypers 1982 for review) This third system originates mainly from the aminergic centers in the brain stem (i e locus coeruleus and subcoeruleus, raphe nuclei, and certain parts of the reticular formation), courses mainly via the ventral part of the lateral funiculus, and terminates directly on motoneurons Since its sites of origin receive an input from limbic structures (viz , amygdala, periaqueductal grey), the third system might particularly provide motivational drive in the execution of movements, and be especially active in circumstances of fight and flight

The study of the above-mentioned aspects of supraspinal motor control can be usefully undertaken in reptiles, since in these animals the reticular formation, in addition to several other brain stem nuclei, forms the most important center for motor behaviour, while a direct corticospinal pathway is lacking Early studies in reptiles based on normal anatomical descriptions (for reviews see Ariens Kappers et al 1936, Nieuwenhuys 1964), as well as the first experimental studies using anterograde and retrograde degeneration techniques (Robinson 1969, Cruce 1975, ten Donkelaar 1976a,b; Cruce and Newman 1981) already demonstrated the presence of several supraspinal descending pathways similar to those in mammals Furthermore a comparable organization of these pathways in a medial and lateral component could be established (ten Donkelaar 1976b) However, the limitations of the retrograde degeneration technique did not allow more extensive studies of the connections between the brain stem and the spinal cord spinal lesions caudal to high thoracic levels failed to elicit chromatolysis in the brain stem As soon as the modern, more sensitive anterograde and retrograde tracer techniques had opened the perspective of further investigations in this field, more detailed information on the precise course and site of termination, and on the localization of the cells of origin of the descending supraspinal pathways came available

In addition to the major descending pathways to the spinal cord already known, spinal projections were demonstrated from several other cell groups in the brain stem, e g the nucleus Edinger-Westphal, the locus coeruleus and subcoeruleus, the nucleus descendens nervi trigemini, the dorsal motor nucleus of the vagus nerve, the dorsal column nuclei, and from the hypothalamus, e g nucleus paraventricularis, nucleus periventricularis hypothalami (ten Donkelaar and de Boer-van Huizen 1978, ten Donkelaar et al 1980, Woodson and Kunzle 1982) Moreover some data on the funicular trajectory of these pathways were obtained (ten Donkelaar et al 1980, Cruce and Newman 1981) Considering the evidence for a basic pattern in the organization of the descending supraspinal pathways in terrestrial vertebrates (ten Donkelaar 1976b, 1982), the evidence for the presence of monoaminergic cell populations in the reptilian brain stem (Parent and Poirier 1971, Parent 1979, Yamamoto et al 1977), and the existence of extensive spinal projections from such cell groups in mammals, it was postulated that part of the pathways from the brain stem to the spinal cord in reptiles would be monoaminergic of nature (ten Donkelaar et al 1980)

This wealth of new data formed the starting point for the present study, focussed on the organization of the descending supraspinal pathways in a lizard, *Varanus exanthematicus* Various modern tracer techniques have been used for further investigations on the origin, course and site of termination of these pathways horseradish peroxidase (HRP), fluorescent tracers ("Fast Blue" and "Nuclear Yellow"), and tritiated leucine ([^{3}H]leucine) In addition immunohistochemistry was used for a study of the distribution of catecholamines, serotonin, and some peptides in the brain stem, the caudal diencephalon, and the spinal cord

The present account starts with a cytoarchitectonic analysis of the cell masses in the brain stem and caudal diencephalon (Chapter 1), which is not only based on normal material, but on certain experimental studies, including the chapters 2 to 6 of this thesis, as well

In Chapter 2 the funicular trajectories of the descending brain stem pathways have been studied, by implanting HRP slow release gels into four different quadrants of the cervical spinal enlargement

Chapters 3 to 5 focus on the distribution of catecholamines (3), serotonin (4), and the peptides substance P, [Leu] and [Met]-enkephalin (5) in the brain stem, caudal diencephalon, and spinal cord The indirect immunofluorescence technique was applied, making use of antibodies to tyrosine hydroxylase, serotonin, and the three mentioned peptides, respectively

Chapter 6 deals with the problem of collateralization of the descending pathways, in order

to answer the following questions. 1) Do the descending brain stem pathways give off collaterals that may influence widely separate levels of the spinal cord ? 2) Are there quantitative differences in the degree of collateralization of different descending brain stem pathways ? 3) Is there evidence for a somatotopic pattern within brain stem projections to the spinal cord ?

In Chapter 7 the spinal projections from the nucleus raphes inferior and the adjacent reticular formation, obtained with the anterograde [^{3}H]leucine tracing technique, will be described

A summary concludes the present study.

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THE CELL MASSES IN THE BRAIN STEM AND CAUDAL DIENCEPHALON OF THE LIZARD VARANUS EXANTHEMATICUS

Introduction

The brain stem as defined here comprises the mesencephalon and the rhombencephalon. It contains the nuclei of origin and termination of most cranial nerves, a well-developed reticular formation, and numerous sensory and motor relay nuclei (e.g. the red nucleus) with their associated ascending and descending connections. The present study deals with the descending pathways to the spinal cord. Since rather extensive spinal projections also arise in the caudal hypothalamus (ten Donkelaar 1982; ten Donkelaar and de Boer-van Huizen 1978; ten Donkelaar et al. 1980; Wolters et al. 1985a), the present atlas comprises a survey of the brain stem and the caudal hypothalamus.

Several studies have dealt with the cytoarchitectonic organization of the reptilian brain stem (de Lange 1913, 1917; Beccari 1923; Shanklin 1930; Frederikse 1931; Tuge 1932; Warner 1935; Ariens Kappers et al. 1936; Stefanelli 1944; Anthony 1970; Cruce and Nieuwenhuys 1974; Molenaar 1977; ten Donkelaar and Nieuwenhuys 1979; Newman and Cruce 1982) and hypothalamus (Senn 1968; Butler and Northcutt 1973; Cruce 1974; Prasada Rao and Subhedar 1977; Northcutt 1978; Prasada Rao et al. 1981; Franzoni and Fasolo 1982). Only one study (ten Donkelaar and Nieuwenhuys 1979) covers the organization of the entire brain stem in a lizard closely related to *Varanus exanthematicus*: i.e. the tegu lizard, *Tupinambis nigropunctatus*. Most of the above-mentioned studies are based on normal material.

The present analysis of the brain stem of *Varanus exanthematicus* is based on Nissl (cresylecht violet) and Kluver-Barrera stained material, but also on data of several experimental studies, as far as they contribute to a further subdivision of the neuronal populations of the brain stem (HRP experiments: e.g. ten Donkelaar 1982; ten Donkelaar et al. 1980; Bangma and ten Donkelaar 1984; ten Donkelaar and de Boer-van Huizen 1981a,b, 1984a; Wolters et al. 1982; Hoogland 1982; Barbas-Henry 1982; Barbas-Henry and Lohman 1984; fluorescent tracer studies: ten Donkelaar and de Boer-van Huizen 1984b; Wolters et al. 1985a; immunohistochemical studies: Wolters et al. 1984, 1985b,c). The topographical relationships of the various cell masses in the brain stem and the caudal diencephalon are illustrated with photographs of representative Nissl stained sections at the left, and the up-to-date delineated cell masses in *Varanus exanthematicus* at the right. In addition the main fiber pathways have been indicated. Following some brief remarks on the gross anatomy, the various cell masses in the brain stem and caudal hypothalamus of *Varanus exanthematicus*, as well as their main afferent and efferent connections, will be described, grouped under the following headings: 1. Somatomotor nuclei; 2. Branchiomotor nuclei; 3. Visceromotor nuclei; 4. Somatosensory nuclei; 5. Viscerosensory nuclei; 6. Vestibular and cerebellar nuclei; 7. Cochlear nuclei and superior olive; 8. Reticular formation and inferior olive; 9. Nuclei of the isthmic region; 10. Mesencephalic nuclei; 11. Hypothalamic nuclei. The last three headings cover only those nuclei not mentioned in one of the preceding paragraphs.

Materials and techniques

The normal material studied consists of four transversely sectioned Nissl stained (cresylecht violet) series and one Kluver-Barrera stained series. In addition, a Nissl and a Kluver-Barrera stained sagittal series were used.

Delineation of the cell masses was performed using the following cytoarchitectural criteria: 1) density and arrangement of the neuronal elements; 2) size and shape of the cell bodies; 3) aspect and distribution of the Nissl substance. Cell sizes were

Figs. 1-2. Photographs of the brain of the lizard *Varanus exanthematicus*, lateral and dorsal view, respectively. (Bar: 2 mm)

measured directly by means of an ocular micrometer, and determined by dividing the sum of the two largest perpendicular diameters by two. As to the cell size, five rather arbitrarily chosen categories of cells were distinguished, following Cruce and Nieuwenhuys (1974): very small cells: 8-12 μm; small cells: 13-19 μm; medium-sized cells: 20-29 μm; large cells: 30-40 μm; very large cells: >40 μm.

For topographical reconstruction of the brain stem in the sagittal and horizontal planes, Nissl stained transverse sections (thickness 15 μm) were matched with a hypothetical midsagittal plane, based on the sagittal series. The cell masses, as delineated in the transverse series, were projected upon the horizontal and sagittal reconstructions of the brain stem. Furthermore all large (30-40 μm) and very large (>40 μm) neurons present in the reticular formation were plotted directly into these reconstructions, using an X-Y plotter (Kipp Instruments). In these drawings (Figs. 17, 18, 22) only one out of five of the large and very large reticular elements has been indicated.

The different fiber sizes, as indicated in the schematic drawings (Figs. 3-16), were obtained from observations made in Klüver-Barrera and Häggqvist material of earlier studies (ten Donkelaar and Nieuwenhuys 1979). The terms small, medium-sized, and coarse fibers are not strictly defined, but generally range from 0 to 3 μm, from 3 to 6 μm, and from 6 to 12 μm in diameter respectively.

Results

Gross anatomy

As in most reptiles, the brain stem of *Varanus exanthematicus* is rather strongly curved (Figs. 1, 17-19). The fossa rhomboidea is covered by a richly vascularized tela choroidea which is attached to the dorsal aspect of the alar plate and to the cerebellum. The cerebellum in *Varanus exanthematicus* is tilted forward, i.e. everted, resulting in a dorsal position of part of the granular layer (see Bangma 1983; ten Donkelaar and Bangma 1985). Its base forms the

roof of the rostralmost part of the fourth ventricle, and continues rostrally into the velum medullare anterius. Laterally the cerebellar peduncles interconnect the cerebellum and the brain stem. Just caudal to the bilateral domes of the large tectum mesencephali the two bulbs of the torus semicircularis are found, hidden under the corpus cerebelli. The most rostral parts of the cerebellum and of the rhombencephalon constitute a narrowed part of the brain stem, which is known as the isthmus rhombencephali. Here both trochlear nerves leave the brain stem at its dorsal side. Further rostrally the fourth ventricle narrows considerably, proceeds diamond-shaped over a very short distance as aqueductus cerebri (Fig. 6), and widens again beneath the tectum mesencephali, as the caudal part of the third ventricle, where both lateral recesses extend into the domes of the tectum (Figs. 4, 5). Rostral to the place where the oculomotor nerves leave the tegmentum mesencephali, the mesodiencephalic junction is marked at the ventral side by the two small, dome-shaped swellings of the mamillary bodies, rostral to which the hypothalamus protrudes ventrally. This part of the diencephalon, which is continuous with the mesencephalic tegmentum, is covered dorsally by the rostral part of the tectum mesencephali (Figs 1, 2, 20-22).

Somatomotor nuclei

All somatomotor nuclei are situated close to the midline. This somatic efferent column comprises the nucleus of the hypoglossal nerve and the centers which supply the external ocular muscles, i.e. the nuclei of the oculomotor nerve, the trochlear nerve and the abducens nerve. In addition to a close topographic relation to the fasciculus longitudinalis medialis (flm), the external eye muscle nuclei are functionally related to the flm by internuclear connections and vestibular afferents coursing via this bundle (Bangma and ten Donkelaar 1983; ten Donkelaar et al. 1985).

The *nucleus nervi oculomotorii* (Fig. 6) surrounds the dorsal and medial confines of the rostralmost part of the flm in the mesencephalon. It consists of large and medium-sized neurons, and can be subdivided into a *pars dorsalis*, which as a sickle-shaped cap lies over the flm, a *pars intermedia* medial to the flm, and a small *pars ventralis*, the rostral part of which is lying ventromedial to the flm, the caudal part merging with the medial side of the pars intermedia. The nervus oculomotorius leaves the mesencephalon in a rostroventrolateral direction.

The *nucleus nervi trochlearis* (Fig. 7) gradually passes over into the nucleus nervi oculomotorii pars dorsalis, and consists of similarly shaped neurons. The caudal pole of this small nucleus reaches the isthmic region, but does not extend beyond the torus semicircularis. The nervus trochlearis leaves the nucleus laterally, hooks around the lateral recess of the fourth ventricle, and crosses the midline in the velum medullare anterius, where it leaves the brain stem.

The *nucleus nervi abducentis* (Fig. 11) lies considerably more caudally, at the level of the entrance of the nervus vestibulocochlearis, immediately lateral to the flm. It consists of medium-sized cells with darkly stained Nissl substance, which can be easily recognized. The nervus abducens leaves the rhombencephalon in ventrolateral direction.

The well-developed *nucleus nervi hypoglossi* (Figs. 15, 16) lies directly lateral to the flm, and consists mainly of large cells. A dorsomedial part and a ventrolateral part can be distinguished, although both parts merge at various levels over the entire length of the nucleus. The caudal part of the nucleus nervi hypoglossi proceeds into the spinal cord, and is continuous with the motoneuronal cell column of the ventral horn. Efferent fibers leave the nucleus in ventral and slightly laterocaudal direction, forming small fiber bundles that leave the brain stem over the entire extent of the nucleus in lateral direction (see also Barbas-Henry and Lohman 1984).

Branchiomotor nuclei

This group comprises the nucleus motorius nervi trigemini, the nucleus motorius nervi facialis, and the nucleus ambiguus. From the latter nucleus efferent fibers join both the glossopharyngeal and the vagus nerve. These nuclei form an almost continuous column in the lateral part of the basal plate, extending from the caudal isthmic level to the middle part of the medulla oblongata.

The *nucleus motorius nervi trigemini* (Fig. 10) can be divided into a small dorsal part, which contains large and a few medium-sized cells, and a large ventral part, consisting of large and very large cells, containing individual strands of Nissl substance. From this nucleus the efferent fibers take a mainly ventrolateral course to join the trigeminal nerve at its ventral side.

The *nucleus motorius nervi facialis* (Fig. 12) consists of large polygonal cells, and extends from the level of the nucleus nervi abducentis to the level of the caudal root of the VIIIth nerve. In Nissl material a dorsomedial and ventrolateral part can be distinguished. From here the efferent fibers course in rostral and dorsomedial direction to the sulcus limitans, ventral to which they bend sharply into the lateral direction (facial genu), coursing ventral to the nucleus vestibularis ventrolateralis before leaving the brain stem (Barbas-Henry 1982).

The *glossopharyngeal part* of the *nucleus ambiguus* (Figs. 13, 14) lies just ventral to the lateral part of the nucleus motorius nervi facialis, and consists mainly of medium-sized cells, so that delineation of both nuclei is not difficult. A retrograde

tracer study in which "Fast Blue" was applied to the glossopharyngeal nerve and "Nuclear Yellow" to the facial nerve, demonstrated that both nuclei show some overlap in rostrocaudal direction (Barbas-Henry and Lohman 1984) Caudally the glossopharyngeal part of the nucleus ambiguus fades at the level of the rostral pole of the nucleus motorius dorsalis nervi vagi The *vagal part* of the *nucleus ambiguus* (Figs 14-16) is not continuous with the glossopharyngeal part, but lies more dorsocaudally, directly lateral to the nucleus nervi hypoglossi Rostrally this nucleus consists of small to medium-sized cells, and is continuous with the nucleus salivatorius inferior Caudally it contains medium-sized and large cells and merges with the grey matter of the first spinal segment It cannot be excluded that this part of the nucleus ambiguus has a general visceromotor (parasympathetic) function in addition to a branchiomotor function The efferent fibers of the nucleus ambiguus course through the brain stem in a similar way as those of the facial nerve

Visceromotor nuclei

So far only two visceromotor (parasympathetic) nuclei could be delimited in reptiles, viz , the nucleus Edinger-Westphal and the nucleus motorius dorsalis nervi vagi Recent HRP data, however, suggest the presence of a nucleus salivatorius superior and a nucleus salivatorius inferior as found in mammals (Barbas-Henry and Lohman 1984)

The small *nucleus Edinger-Westphal* (Fig 5), also called accessory nucleus nervi oculomotorii, consists of small and medium-sized spindle-shaped cells which lie directly dorsorostral to the nucleus nervi oculomotorii pars dorsalis Its efferent fibers mainly course with the oculomotor nerve, but in reptiles, as in mammals (Kuypers and Maisky 1975, Loewy and Saper 1978, Loewy et al 1978, Castiglioni et al 1978), a modest spinal projection has also been demonstrated (ten Donkelaar and de Boer-van Huizen 1978, ten Donkelaar et al 1980) It should be noted, however, that it is uncertain whether this spinal projection arises from the preganglionic parasympathetic neurons which constitute the nucleus Edinger-Westphal (Akert et al 1980)

The *nucleus salivatorius superior* (Fig 12) and *nucleus salivatorius inferior* (Figs 13, 14) cannot easily be delineated in normal material HRP experiments, however, revealed the presence of these nuclei, consisting of medium-sized and small spindle-shaped cells, just lateral to the nucleus motorius nervi facialis and the glossopharyngeal part of the nucleus ambiguus, respectively (Barbas-Henry 1982, Barbas Henry and Lohman 1984) These presumably parasympathetic nuclei extend over the entire length of both branchiomotor nuclei, except for the rostralmost part of the nucleus motorius nervi facialis Both nuclei merge and show a certain overlap Their efferent fibers join the facial nerve and the glossopharyngeal nerve Caudally, the nucleus salivatorius inferior is continuous with the relatively small-celled rostral vagal part of the nucleus ambiguus

The *nucleus motorius dorsalis nervi vagi* (Figs 14-16) is a slender, but distinct nucleus, situated just lateral to the central canal and to the ventral part of the caudal one-third of the fossa rhomboidea It consists of densely packed small and medium-sized cells of which the Nissl substance is darkly stained Caudally the nucleus is continuous with the intermediate zone of the spinal cord Efferent fibers course in dorsolateral direction into the root of the vagus nerve In addition, a spinal projection from this nucleus has been established (ten Donkelaar and de Boer-van Huizen 1978, ten Donkelaar et al 1980, Cruce and Newman 1981, Wolters et al 1982)

General somatosensory nuclei

The *nucleus mesencephalicus nervi trigemini* (Figs 4-6) is formed by a rather compact group of mainly large round and ellipsoid cells just dorsal to the aqueductus cerebri, which flattens out more rostrally throughout the medialmost part of the periventricular layer of the tectum mesencephali The nucleus also comprises some scattered cells of the same type, lying more laterally in this layer The nucleus has been shown to mediate proprioceptive information from the muscles of mastication (Desole et al 1970) Most of the efferent fibers leave the nucleus caudally, curve dorsolaterally around the nucleus centralis of the torus semicircularis at the level of the isthmo-mesencephalic junction, and take a more ventral course at the level of the cerebellar peduncle, just ventrolateral to the sulcus limitans, where one component gradually joins the trigeminal nerve root, and the other courses in the direction of the trigeminal motor nucleus (Barbas-Henry 1984), and continues more caudally (see also Dacey 1982)

The *nucleus princeps nervi trigemini* (Fig 10) is a horizontally flattened nucleus, consisting of medium-sized and small cells, overlying the brain stem part of the trigeminal nerve root It extends from the caudal isthmic level to the caudal limit of the entering trigeminal nerve The lateral part of this nucleus curves ventrally where it is traversed by trigeminal fiber bundles A modest spinal projection from the nucleus princeps nervi trigemini has been demonstrated (Woodson and Kunzle 1982, Wolters et al 1985a), as well as a more distinct ascending projection to or beyond the rostral mesencephalon (ten Donkelaar and de Boer-van Huizen 1981b, 1984b, Hoogland 1982)

Ventral to the entering trigeminal nerve, the *nucleus descendens nervi trigemini* (Figs 11 16) is present as a ventral and caudal continuation of the

nucleus princeps nervi trigemini It extends from the level of the trigeminal nerve root throughout the rhombencephalic alar plate, and merges with the dorsal horn of the spinal grey matter From rostral to caudal this nucleus gradually shifts from a ventrolateral to a dorsal position in the alar plate, immediately lateral to the nucleus funiculi dorsalis The nucleus descendens nervi trigemini consists mainly of medium sized and small cells, which are more loosely arranged in its caudal part The nucleus descendens nervi trigemini cannot easily be delineated over its caudal extent in normal material Experimental studies, however, have revealed the exact course and site of termination of the descending trigeminal tract (Barbas-Henry 1984), thus indicating the position of the nucleus descendens nervi trigemini as well In addition to the main input from the descending trigeminal tract, this nucleus receives afferent fibers from the solitary tract, converging on its mediodorsal pole They constitute the somatosensory components of the facial, glossopharyngeal and vagus nerves (Jacobs 1979, Barbas Henry 1982, 1984, Barbas-Henry and I ohman 1984)

The *nucleus funiculi dorsalis* (Figs 14-16) is an elongated, slender nucleus, consisting of loosely arranged small and very small cells, lying in the very top of the alar plate It extends from the caudal pole of the cochlear nuclear complex to the transition from medulla to spinal cord, where it fades in the dorsal funiculus A dorsal indentation makes it possible to distinguish a medial and a lateral part of this nucleus, probably homologous to the mammalian nuclei gracilis and cuneatus, respectively As in mammals, a somatotopic organization of the ascending spinal fibers projecting to the nucleus funiculi dorsalis has been demonstrated in various reptiles (Kruger and Witkovsky 1961, Goldby and Robinson 1962, Ebbesson 1967, 1969, Joseph and Whitlock 1968, Kusuma and ten Donkelaar 1980, Kunzle and Woodson 1983) The main output of this nucleus is represented by the medial lemniscus, a decussating ascending projection to the ventrolateral thalamic area, the nucleus centralis of the torus semicircularis and the inferior olive (Ebbesson 1978) The medial lemniscus can be found, throughout the rhombencephalon, as a distinct thin-fibered bundle near the ventral brain stem surface, close to the midline (Figs 7-16) A modest spinal projection of the nucleus funiculi dorsalis has also been demonstrated (ten Donkelaar and de Boer van Huizen 1978, ten Donkelaar et al 1980, Woodson and Kunzle 1982)

Viscerosensory nuclei

The *nucleus tractus solitarii* (Figs 14-16) is an elongated nucleus immediately lateral to the fourth ventricle, extending along the caudal one-third of the fossa rhomboidea, caudally merging with the dorsal horn of the first spinal segment Caudal to the obex, the dorsal parts of the nucleus on each side of the brain stem merge, thus forming the *nucleus of the commissura infima* (caudal to the level of Fig 16) The nucleus tractus solitarii consists of small cells, loosely arranged in a triangular area, as viewed in transverse sections Ventrally, the nucleus motorius dorsalis nervi vagi protrudes within the confines of this nucleus Dorsolaterally, the nucleus is delineated over its entire length by the solitary tract, which extends even more rostrally From this tract the nucleus receives its main input, i e fibers from the glossopharyngeal and vagus nerves (Barbas-Henry and Lohman 1984) and from the facial nerve (Jacobs 1979, Barbas Henry 1982) Most parts of the nucleus tractus solitarii receive a dense serotonergic innervation (Wolters et al 1985b), whereas its medial part receives a [Leu]- and a [Met]-enkephalinergic innervation and contains a high density of substance P-immunoreactive varicosities (Wolters et al 1985c) Neurons in the nucleus tractus solitarii have been shown to contain catecholamines (Wolters et al 1984) and various peptides (Wolters et al 1985c)

A projection from the nucleus tractus solitarii to the *parabrachial region* (Figs 7, 8), as demonstrated in mammals (Norgren and Leonard 1973, Mantyh and Hunt 1984), has been suggested also for reptiles (Shanklin 1930, Barnard 1936, ten Donkelaar and de Boer-van Huizen 1981b) The presence of a high density of substance P- and [Met]- and [Leu]-enkephalin immunoreactive terminals (Wolters et al 1985c), as well as a modest catecholaminergic innervation (Wolters et al 1984) in the parabrachial region of *Varanus exanthematicus*, corroborate this suggestion This region also receives a dense serotonergic innervation (Wolters et al 1985b) In addition to this ascending projection from the nucleus tractus solitarii, a modest spinal projection exists (ten Donkelaar et al 1980, Cruce and Newman 1981, Wolters et al 1982, 1985a)

Vestibular and Cerebellar nuclei

In reptiles the vestibular and cerebellar nuclei form a more or less continuous complex, which occupies almost the entire ventral zone of the rhombencephalic alar plate (Cruce and Nieuwenhuys 1974) For a detailed account of the cerebellum in various reptiles, including *Varanus exanthematicus*, we refer to Bangma (1983) and ten Donkelaar and Bangma (1985) The reptilian cerebellar cortex is composed of the three typical layers, i e the molecular, Purkyne cell and granular layer (Figs 7-10) Two cerebellar nuclei, viz the nucleus cerebellaris medialis and the nucleus cerebellaris lateralis, can be distinguished

The *nucleus cerebellaris medialis* (Figs 8, 9) is located in the basal part of the corpus cerebelli, immediately dorsolateral to the flattened fourth ventri-

cle, and extends from the level just caudal to the trochlear nerve root to the caudal border of the cerebellar peduncle It consists of scattered large to medium sized oval cells that can easily be recognized and delineated from the surrounding nuclei

The *nucleus cerebellaris lateralis* (Figs 9, 10), which is directly ventrolateral to the nucleus cerebellaris medialis, mainly contains medium sized and small cells It accompanies the nucleus cerebellaris medialis over the caudal two-third of its extent, but extends slightly more caudally Rostrally the nucleus cerebellaris lateralis merges with the parabrachial region

The main input to the medial and lateral cerebellar nuclei arises from the medial zone and a caudolateral area of the Purkyne cell layer, respectively (Bangma and ten Donkelaar 1984) The output of the nucleus cerebellaris medialis consists mainly of descending contralateral projections via the fasciculus uncinatus to the vestibular nuclear complex, to the medullary reticular formation (Bangma et al 1984), and to the spinal cord (ten Donkelaar et al 1980, Wolters et al 1982, 1985a, Woodson and Kunzle 1982, Bangma et al 1984) A small ascending projection from the nucleus cerebellaris medialis was also found (Bangma et al 1984) From the nucleus cerebellaris lateralis mainly ascending projections arise to the contralateral red nucleus, nucleus interstitialis of the flm, diencephalon, and probably also the telencephalon (Lohman and van Woerden-Verkley 1978, ten Donkelaar and de Boer-van Huizen 1981b, Bangma et al 1984) A smaller bilateral descending projection from the nucleus cerebellaris lateralis to the vestibular nuclear complex, as well as a modest contralateral projection to the reticular formation and the spinal cord, have been demonstrated (ten Donkelaar and de Boer-van Huizen 1978, ten Donkelaar et al 1980, Bangma et al 1984)

Caudally the cerebellar nuclei border on the vestibular nuclear complex The reptilian vestibular nuclear complex is usually divided into at least five nuclei (Weston 1936), i e nucleus vestibularis dorsolateralis, nucleus vestibularis ventrolateralis, nucleus vestibularis tangentialis, nucleus vestibularis ventromedialis, and nucleus vestibularis descendens In turtles, in addition a nucleus vestibularis superior can be distinguished (Weston 1936, Cruce and Nieuwenhuys 1974, Miller and Kasahara 1979, ten Donkelaar and Nieuwenhuys 1979, Bangma et al 1983)

The most rostral nucleus of the vestibular nuclear complex, i e the small-celled *nucleus vestibularis dorsolateralis* (Fig 10), extends from the caudal half of the cerebellar peduncle to the level of the trigeminal nerve root

Caudally the nucleus vestibularis dorsolateralis merges with the *nucleus vestibularis tangentialis* (Figs 11, 12), consisting of mainly medium-sized and some large cells, intercalated among the entering fibers of the vestibular nerve root

Ventromedial to the latter nucleus the *nucleus vestibularis ventrolateralis* (Figs 11, 12) is found This most conspicuous nucleus of the vestibular nuclear complex, with its very large and large polygonal cells in which distinct strands of Nissl substance are present, is considered homologous to the mammalian nucleus of Deiters (Robinson 1969, ten Donkelaar 1976b, 1982) It extends from the middle of the trigeminal nerve root to the middle of the vestibular nerve root From the nucleus vestibularis ventrolateralis an extensive, predominantly ipsilateral projection to the spinal cord arises, i e the lateral vestibulospinal tract This tract can be traced easily in Kluver Barrera material (Figs 11-16), takes a ventrolateral position more caudally in the rhombencephalon, and shifts medially into the ventral funiculus in the first spinal segments

The *nucleus vestibularis ventromedialis* (Figs 12, 13) is found immediately ventrolateral to the sulcus limitans It consists of loosely arranged medium-sized and some large cells, and extends from the middle of the vestibular nerve root to the caudal third of the fossa rhomboidea, where it is closely related to the nucleus tractus solitarii

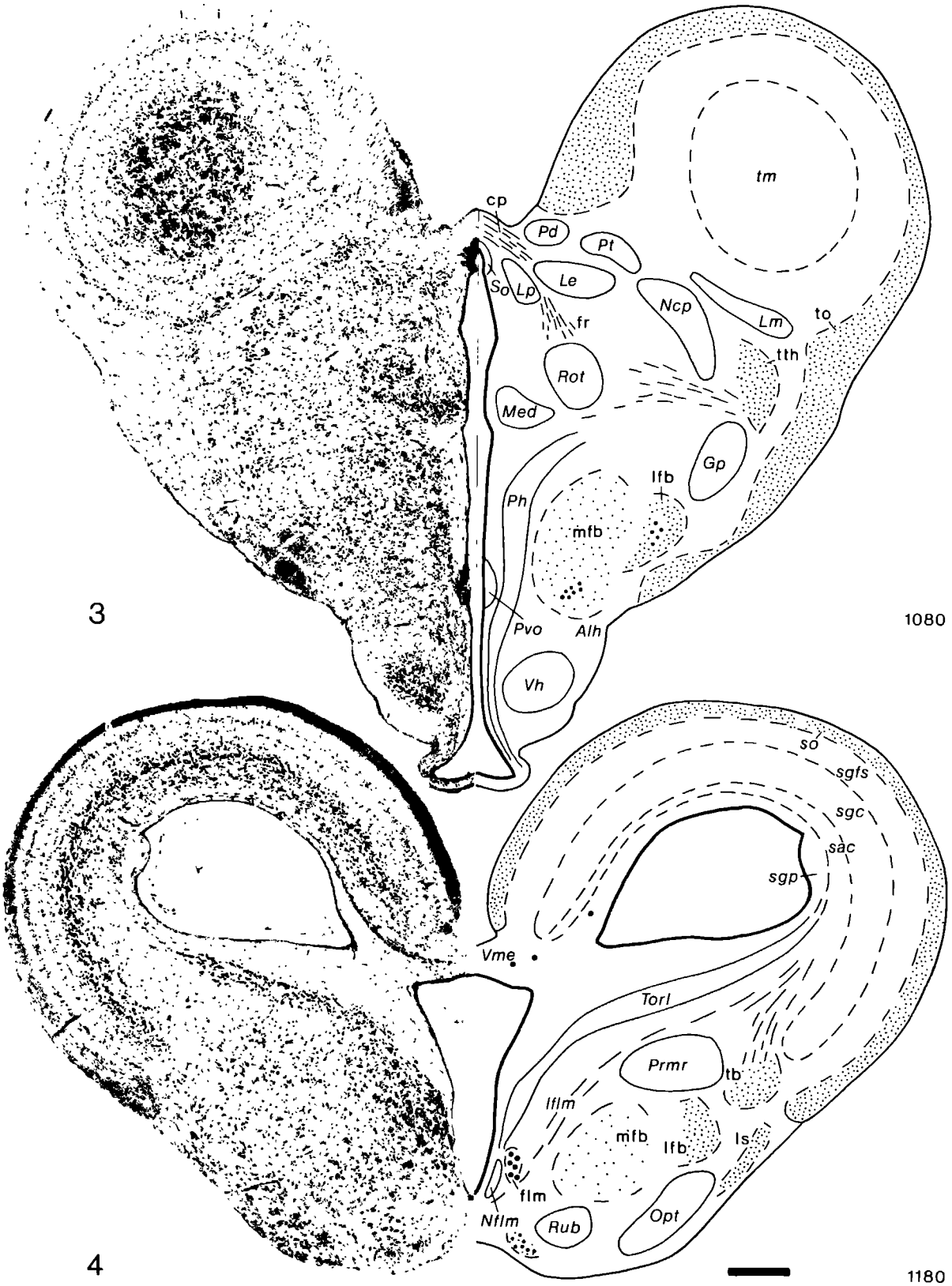
The *nucleus vestibularis descendens* (Figs 13-16), consisting of small and medium-sized cells, borders rostrally on the nucleus vestibularis ventrolateralis, and extends throughout the center of the alar plate of the caudal rhombencephalon, where it fades just caudal to the obex Both the nucleus vestibularis descendens and the nucleus vestibularis ventromedialis give rise to a mainly contralateral spinal projection, which courses via the flm, thus constituting the medial vestibulospinal tract, and continues into the medial part of the ventral funiculus of the spinal cord (ten Donkelaar and de Boer-van Huizen 1978, 1984a, ten Donkelaar et al 1980, Wolters et al 1982, Woodson and Kunzle 1982, Bangma and ten Donkelaar 1983, Newman et al 1983)

Abbreviations used in the figures

Alh	area lateralis hypothalami
Amb	nucleus ambiguus
AmbX	vagal part of the nucleus ambiguus
bc	brachium conjunctivum
c	substantia nigra, pars compacta
cb	cerebellum
Cerl	nucleus cerebellaris lateralis
Cerm	nucleus cerebellaris medialis
Cm	corpus mamillare
Coa	nucleus cochlearis angularis

Codm	nucleus cochlearis dorsalis magnocellularis
Colm	nucleus cochlearis laminaris
cp	commissura posterior
EW	nucleus Edinger-Westphal
fd	funiculus dorsalis
flm	fasciculus longitudinalis medialis
fpd	fasciculus predorsalis
fr	fasciculus retroflexus
Fun	nucleus funiculi dorsalis
Gc	griseum centrale
gl	lamina granularis cerebelli
Gp	nucleus geniculatus pretectalis
Ico	nucleus intercollicularis
Iflm	nucleus interstitialis of the flm
IIId	nucleus nervi oculomotorii, pars dorsalis
IIIi	nucleus nervi oculomotorii, pars intermedia
IIIv	nucleus nervi oculomotorii, pars ventralis
Ipd	nucleus interpeduncularis, pars dorsalis
Ipv	nucleus interpeduncularis, pars ventralis
Ism	nucleus isthmi, pars magnocellularis
Isp	nucleus isthmi, pars parvocellularis
IV	nucleus nervi trochlearis
Lc	locus coeruleus
Le	nucleus lentiformis thalami, pars extensa
lfb	lateral forebrain bundle
Ll	nucleus lemnisci lateralis
ll	lemniscus lateralis
Lm	nucleus lentiformis mesencephali
lm	lemniscus medialis
Lp	nucleus lentiformis thalami, pars plicata
ls	lemniscus spinalis
Med	nucleus medialis
mfb	medial forebrain bundle
ml	lamina molecularis cerebelli
Ncp	nucleus of the commissura posterior
Nflm	nucleus of the flm
nIII	nervus oculomotorius
nIV	nervus trochlearis
nV	nervus trigeminus
nVI	nervus abducens
nVIII	nervus vestibulocochlearis
nX	nervus vagus
nXII	nervus hypoglossus
Oli	oliva inferior
Ols	oliva superior
Opt	nucleus opticus tegmenti (= nucleus of the basal optic root)
Pb	parabrachial region
Pd	nucleus posterodorsalis
Ph	nucleus periventricularis hypothalami
Phg	nucleus prepositus hypoglossi
Pl	Purkyne cell layer
Prmc	nucleus profundus mesencephali, pars caudalis
Prmr	nucleus profundus mesencephali, pars rostralis
Pt	nucleus pretectalis
Pvo	paraventricular organ
r	substantia nigra, pars reticulata
Rai	nucleus raphes inferior
Rasl	nucleus raphes superior, pars lateralis
Rasm	nucleus raphes superior, pars medialis
Ri	nucleus reticularis inferior
Ril	nucleus reticularis inferior, pars lateralis
Ris	nucleus reticularis isthmi
Riv	nucleus reticularis inferior, pars ventralis
Rm	nucleus reticularis medius
Rml	nucleus reticularis medius, pars lateralis
Rot	nucleus rotundus
Rs	nucleus reticularis superior
Rsl	nucleus reticularis superior, pars lateralis
Rub	nucleus ruber
rusp	tractus rubrospinalis
rVme	ramus mesencephalicus nervi trigemini
sac	stratum album centrale
sgc	stratum griseum centrale
sgfs	stratum griseum et fibrosum superficiale
sgp	stratum griseum periventriculare
Sn	substantia nigra
So	subcommissural organ
so	stratum opticum
Sol	nucleus tractus solitarii
spcd	tractus spinocerebellaris dorsalis
spcv	tractus spinocerebellaris ventralis
Si	nucleus salivatorius inferior
Ss	nucleus salivatorius superior
tb	tractus tectobulbaris
tm	tectum mesencephali
to	tractus opticus
Torc	nucleus centralis, torus semicircularis
Torl	nucleus laminaris, torus semicircularis
tsol	tractus solitarius
tth	tractus tectothalamicus
tVds	tractus descendens nervi trigemini
Vds	nucleus descendens nervi trigemini
Vedl	nucleus vestibularis dorsolateralis
Veds	nucleus vestibularis descendens
vespl	tractus vestibulospinalis lateralis
Vetg	nucleus vestibularis tangentialis
Vevl	nucleus vestibularis ventrolateralis
Vevm	nucleus vestibularis ventromedialis
Vh	nucleus ventralis hypothalami
VI	nucleus nervi abducentis
VIImd	nucleus motorius nervi facialis, pars dorsalis
VIImv	nucleus motorius nervi facialis, pars ventralis
Vmd	nucleus motorius nervi trigemini, pars dorsalis
Vmv	nucleus motorius nervi trigemini, pars ventralis
Vme	nucleus mesencephalicus nervi trigemini
Vpr	nucleus princeps nervi trigemini
Vta	ventral tegmental area
X	nucleus motorius dorsalis nervi vagi
XII	nucleus nervi hypoglossi

Figs. 3-16. Representative transverse sections through the brain stem and the caudal diencephalon of a lizard, *Varanus exanthematicus* photomicrographs of the Nissl-stained sections at the left, in the mirrored drawings at the right the delineation of the various cell masses has been indicated, based on the Nissl-stained series, as well as the most prominent fiber tracts, based on Haggqvist and Kluver-Barrera stained series Italic and straight characters indicate names of nuclei and fiber bundles, respectively Certain nuclei, based on experimental data but not clearly distinguishable in Nissl material, have been indicated by broken lines Numbers at the right indicate the levels of the sections, as indicated in the sagittal and horizontal reconstructions (Figs 17-22) (Bar 0 5 mm)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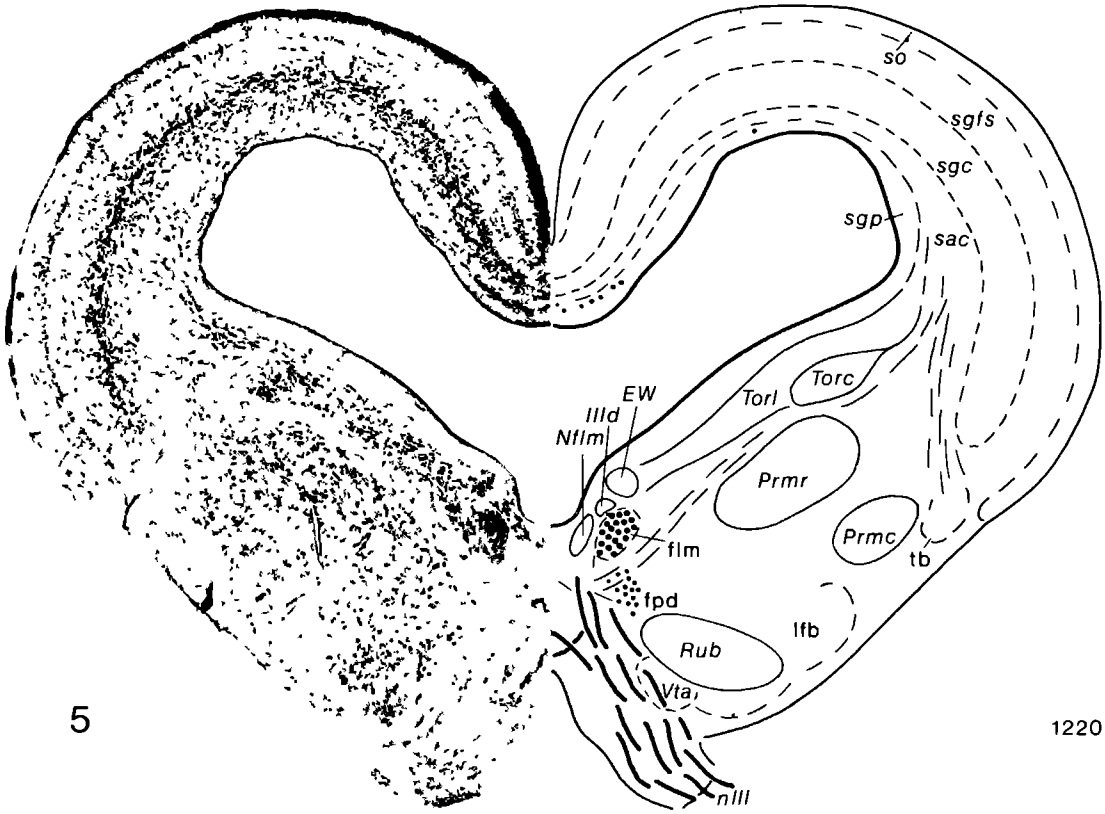
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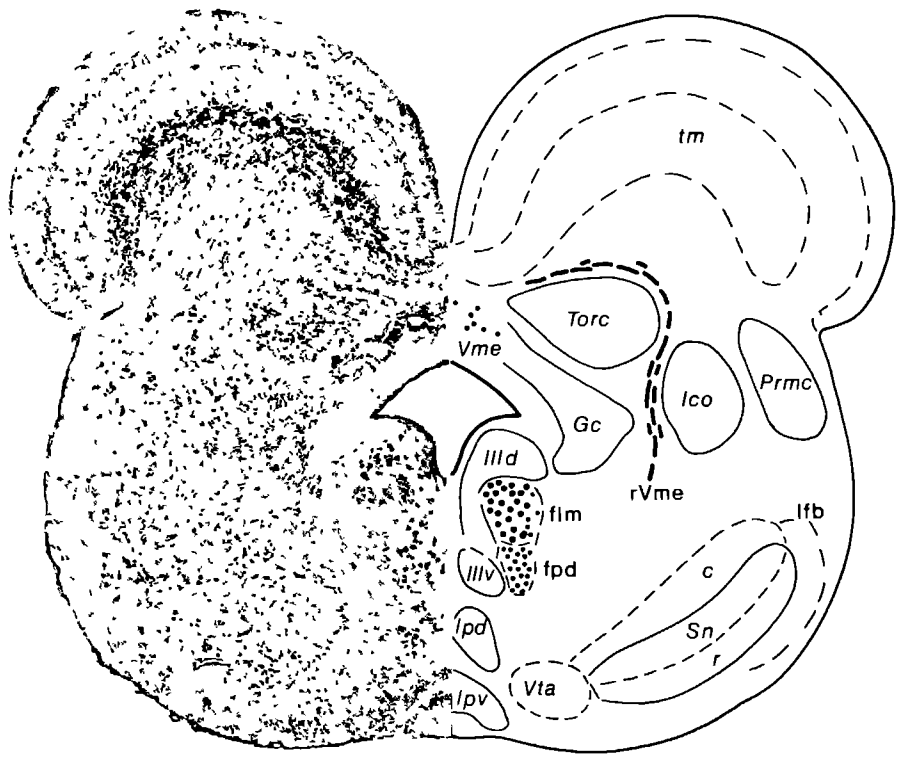
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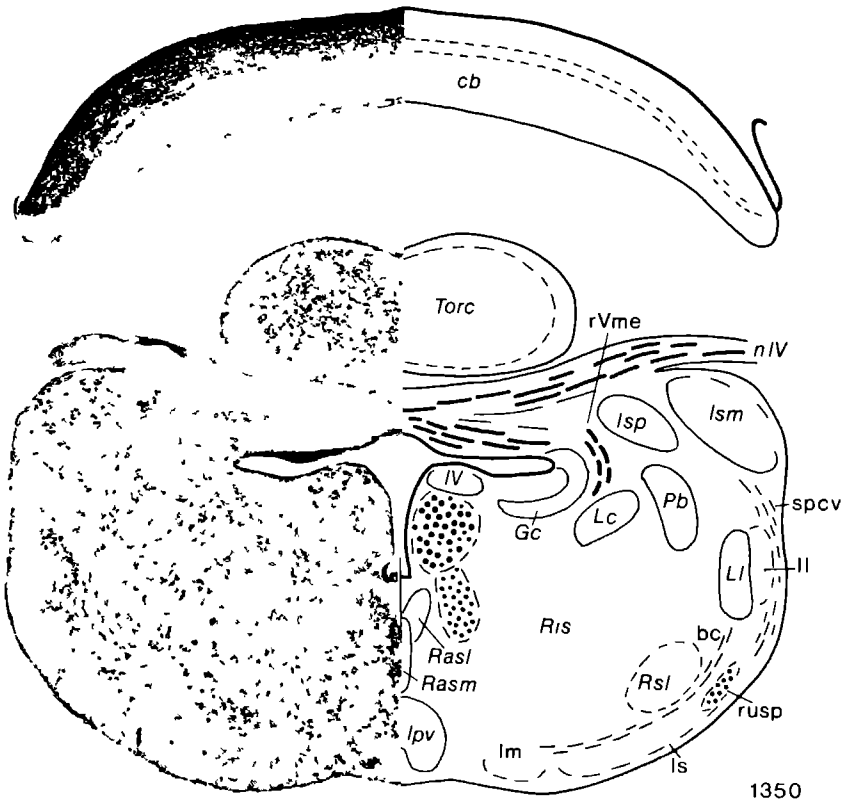
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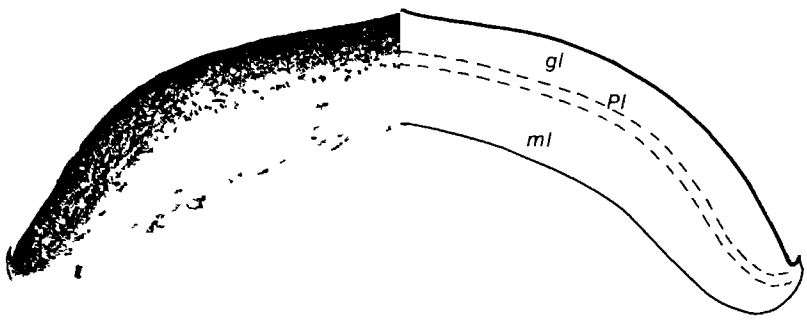
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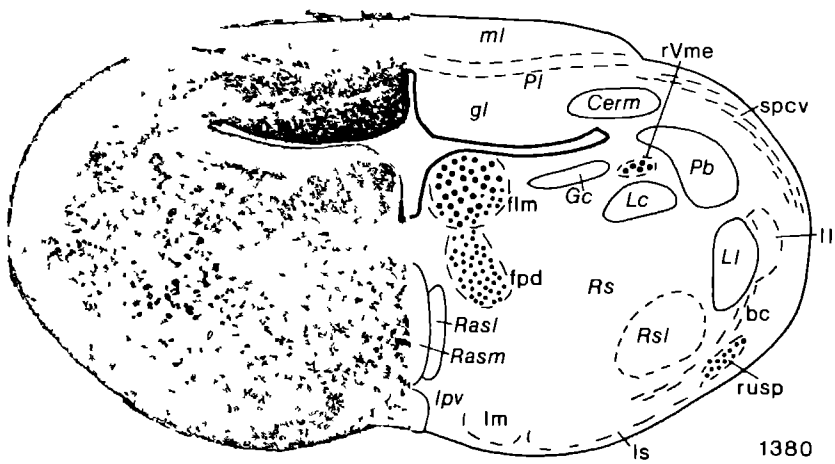
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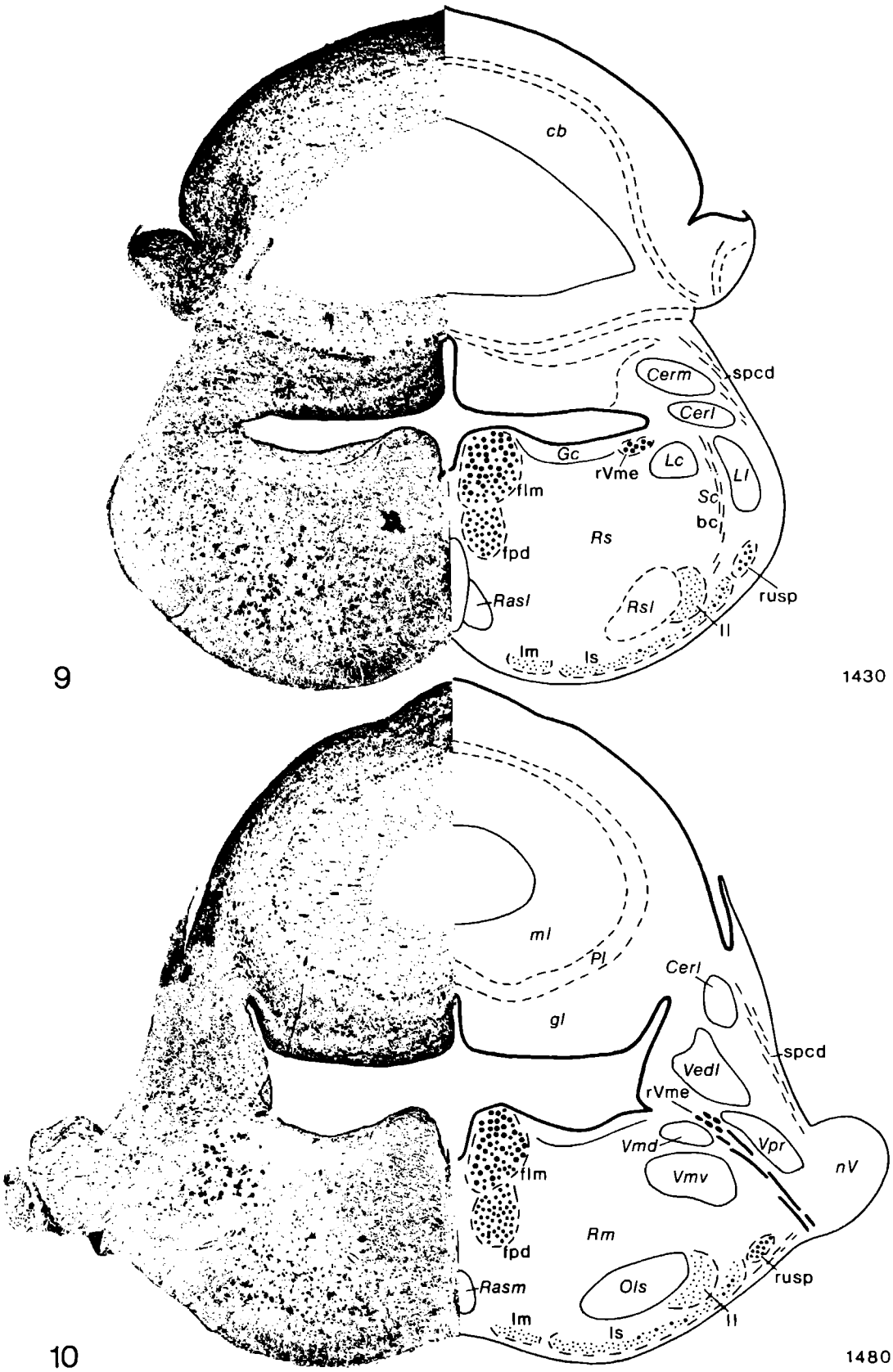
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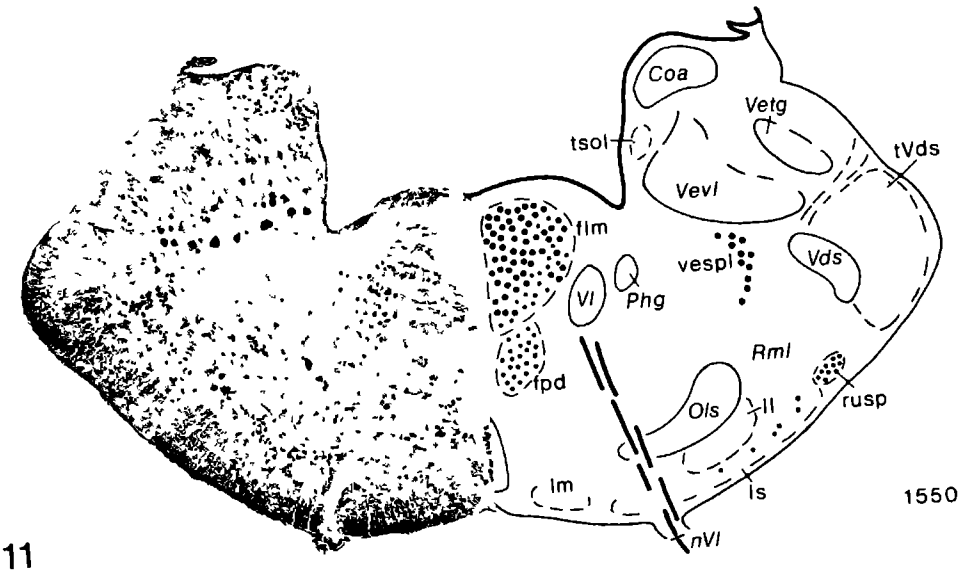
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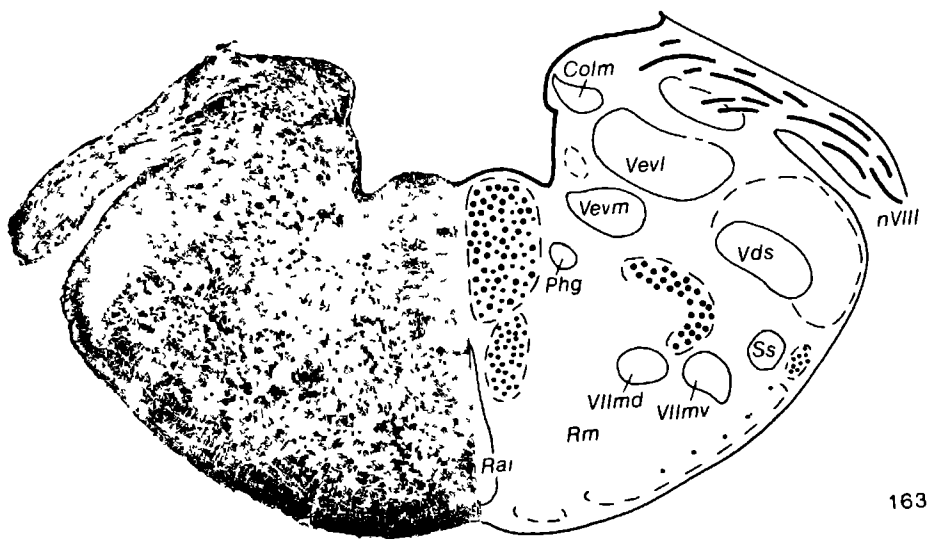
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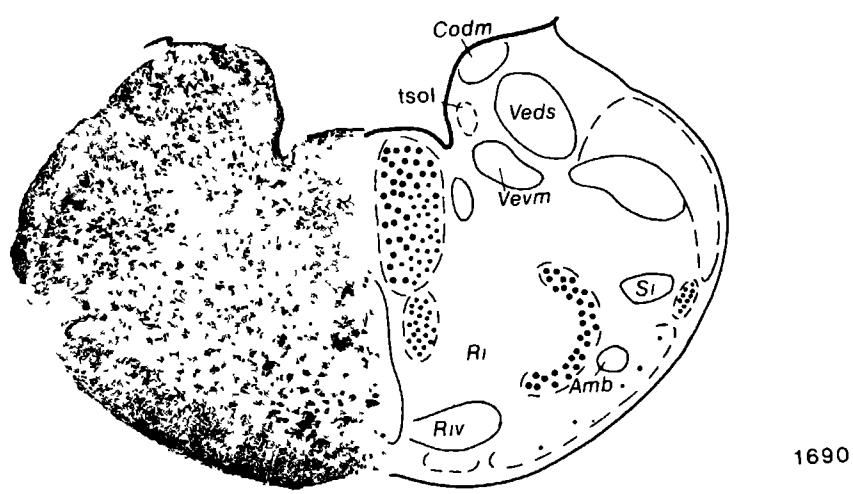
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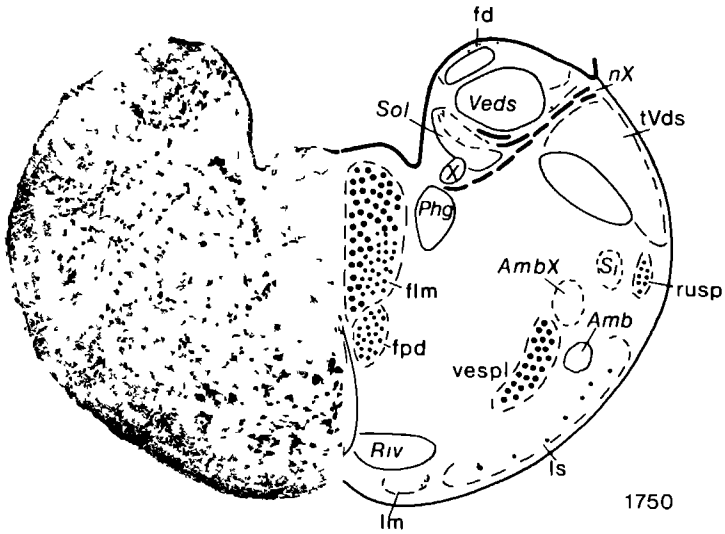

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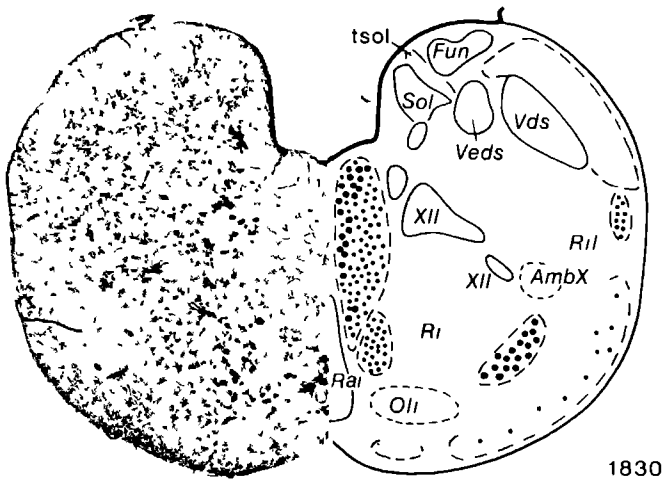

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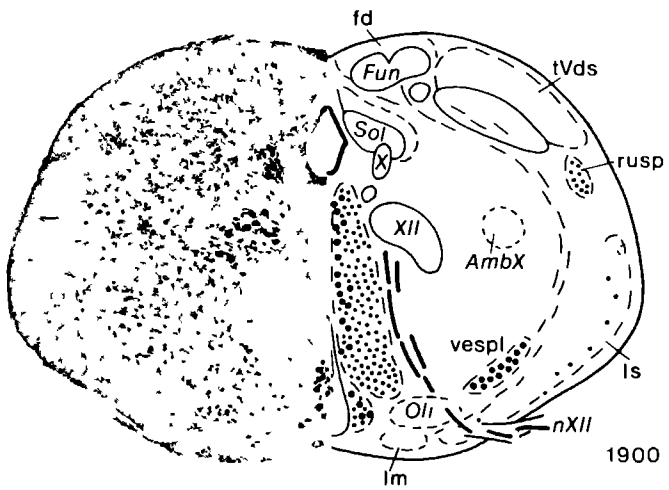


1900

Closely related to the vestibular nuclear complex is the *nucleus prepositus hypoglossi* (Figs. 11-16). First described in various reptiles as the perihypoglossal nuclear complex (Bangma and ten Donkelaar 1982), the localization of the main part of this structure between the nucleus nervi abducentis and the nucleus nervi hypoglossi, its spinal afferents (Ebbesson 1967, 1969; his nucleus parvocellularis medialis), cerebellar projections (Bangma and ten Donkelaar 1982), and projections to the nuclei which innervate the external eye muscles (Bangma and ten Donkelaar 1983; ten Donkelaar et al. 1985) strongly suggest the name nucleus prepositus hypoglossi.

The cochlear nuclei and the superior olive

Three cochlear nuclei can be distinguished in *Varanus exanthematicus*, situated in the acoustic tubercle, i.e. the dorsomedial part of the alar plate at the level of the entrance of the VIIIth nerve.

The *nucleus cochlearis angularis* (Fig. 11) is the most rostral and the largest of the cochlear nuclei in *Varanus exanthematicus*. This distinct nucleus consists of loosely arranged small and some medium-sized cells, and extends rostrally to the level of the cerebellar peduncle.

The *nucleus cochlearis dorsalis magnocellularis* (Fig. 13) is the caudal cochlear nucleus, occupying a dorsal position in the acoustic tubercle. It consists of densely packed mainly very small and small cells, and some medium-sized elements. A lateral magnocellular cochlear nucleus, as distinguished by Miller (1975) in a great variety of lizards, is absent in *Varanus exanthematicus*.

A small *nucleus cochlearis laminaris* (Fig. 12), however, can easily be delineated as a narrow strand of medium-sized and small cells in the ventral part of the region between the nucleus cochlearis angularis and the nucleus cochlearis dorsalis magnocellularis.

A well developed *superior olive* (Figs. 10, 11) is found in the middle of the basal plate as a cluster of small to medium-sized cells. It extends from the caudal isthmic level to the root of the abducens nerve, taking a more dorsal position at its caudal pole. In addition to a projection from the cochlear nuclei, the superior olive has been demonstrated to recieve an input from the mesencephalic tectum (ten Donkelaar 1976b). Its main projection courses via the lateral lemniscus to the nucleus centralis of the torus semicircularis (Foster and Hall 1978).

The reticular formation

Our knowledge of the reticular formation of the reptilian brain stem is largely based on the work of van Hoevell (1911), Beccari (1922), Tuge (1932), and Stefanelli (1941, 1944). The reptilian reticular formation can be subdivided in three longitudinal zones (Cruce and Nieuwenhuys 1974; ten Donkelaar and Nieuwenhuys 1979): 1) a median zone which remains restricted to the rhombencephalon, comprising the unpaired raphe nuclei (Figs. 18, 19, 22); 2) a medial large-celled zone, comprising the magnocellular reticular nuclei in the rhombencephalon and mesencephalon (Figs. 17, 22); 3) a lateral small-celled zone.

The median zone comprises two condensations, the nucleus raphes superior and the nucleus raphes inferior. The *nucleus raphes superior* consists of a *pars medialis* (Figs. 7-10, 18, 22), formed by two vertical rows of closely packed medium-sized cells near the midline, and a *pars lateralis* (Figs. 7-9, 19, 22), which consists of small and very small loosely arranged cells which border on the rostral one-third of the pars medialis. The nucleus raphes superior pars medialis extends from the rhombomesencephalic junction to the caudal isthmic level, where it shifts dorsally over the rostral pole of the nucleus raphes inferior. Nearly all neurons in the nucleus raphes superior pars medialis contain serotonin (Wolters et al. 1985b).

The *nucleus raphes inferior* (Figs. 11-16, 18, 22) extends throughout the rhombencephalon as a vertical strip consisting mainly of large polygonal neurons, but also small, medium-sized and very large elements are present. In the middle one-third of the nucleus raphes inferior, where very large cells are particularly abundant, this nucleus cannot sharply be delineated from the adjacent magnocellular reticular zone. Retrograde tracer studies in *Varanus exanthematicus* demonstrated ascending projections from the nucleus raphes superior and rostral nucleus raphes inferior to or beyond the rostral mesencephalon (Hoogland 1982; ten Donkelaar and de Boer-van Huizen 1981b, 1984b), and descending projections from the nucleus raphes inferior to the spinal cord (ten Donkelaar et al. 1980; Wolters et al. 1982, 1985a).

The medial magnocellular reticular zone constitutes the most extensive part of the reticular formation, the rhombencephalic part of which can be subdivided into three main parts, the nucleus reticularis superior, nucleus reticularis medius, and nucleus reticularis inferior. This nomenclature was introduced by Ariëns Kappers et al. (1936), based on the studies of van Hoevell (1911), Beccari (1922), and Tuge (1932). In a variety of reptiles these nuclei were further subdivided by Newman and Cruce (1982). Their terminology was adopted, for the greater part, in the present account.

The rostralmost part of the magnocellular reticular field is formed by the *nucleus interstitialis of the flm* (Beccari 1923; Tuge 1932), which consists of scattered large and very large cells (Figs. 17, 22) in the medial part of the rostral mesencephalic tegmentum (Figs. 4, 5). Rostrally, the nucleus interstitialis of the flm merges with the nucleus periventricularis hypothalami, caudally it fades at

the level of the oculomotor nerve root A mainly ipsilateral spinal projection from the nucleus interstitialis of the flm has been demonstrated (Robinson 1969, ten Donkelaar et al 1980; Cruce and Newman 1981, ten Donkelaar 1982, Newman et al 1983, ten Donkelaar and de Boer-van Huizen 1984a, Wolters et al 1982, 1985a)

In the caudal part of the tegmentum mesencephali the *nucleus reticularis isthmi* (Stefanelli 1941, 1944) is found (Figs 6, 7), situated between the flm and the substantia nigra (ten Donkelaar et al 1980, Wolters et al 1985c) This small-celled area is probably comparable to the nucleus reticularis diffusus of Tuge (1932), and continues into the most rostral part of the rhombencephalon

The nucleus reticularis superior as designated by Ariens Kappers et al (1936), i e the pretrigeminal part of the medial magnocellular reticular zone, can be subdivided into a *nucleus reticularis superior* proper (Figs 8, 9), comprising a small-celled ventromedial and a medium-sized to large-celled dorsolateral part, and a *nucleus reticularis superior pars lateralis* (Figs 7-9), consisting of loosely arranged medium-sized and large cells in the ventrolateral isthmic tegmentum The latter subnucleus can be considered homologous to the mammalian ventrolateral pontine tegmentum, considering its predominantly contralateral, extensive spinal projection via the dorsal part of the lateral funiculus (ten Donkelaar and de Boer-van Huizen 1978, ten Donkelaar et al 1980, Wolters et al 1982, 1985a) The nucleus reticularis superior has extensive spinal projections via the flm (ten Donkelaar 1976a,b, ten Donkelaar et al 1980, Cruce and Newman 1981, Wolters et al 1982, 1985a, Newman et al 1983, ten Donkelaar and de Boer-van Huizen 1984a) Both nucleus reticularis superior and nucleus reticularis superior pars lateralis extend from the rostral isthmic level to the level of the rostral fibers of the trigeminal nerve root A small-celled ventromedial part of the nucleus reticularis superior has a mainly ipsilateral ascending projection to or beyond the hypothalamus (ten Donkelaar and de Boer-van Huizen 1981b, 1984b)

The *nucleus reticularis medius* (Figs 10, 12, 17, 22) consists of medium-sized to very large cells, and extends from the level of the trigeminal nerve root to the level of the caudal fibers of the VIIIth nerve root A distinct bilateral spinal projection from the nucleus reticularis medius via the flm (ten Donkelaar et al 1976a,b, ten Donkelaar et al 1980, Cruce and Newman 1981, Newman et al 1983, ten Donkelaar and de Boer-van Huizen 1984a, Wolters et al 1982, 1985a), as well as ascending projections (ten Donkelaar and de Boer-van Huizen 1981b, 1984b) were demonstrated

The nucleus reticularis inferior as defined by Ariens Kappers et al (1936), which extends from the level of the VIIIth nerve root to the first spinal segment, consists of medium-sized to very large cells, and comprises a nucleus reticularis inferior proper and a ventral part, i e the nucleus reticularis inferior pars ventralis

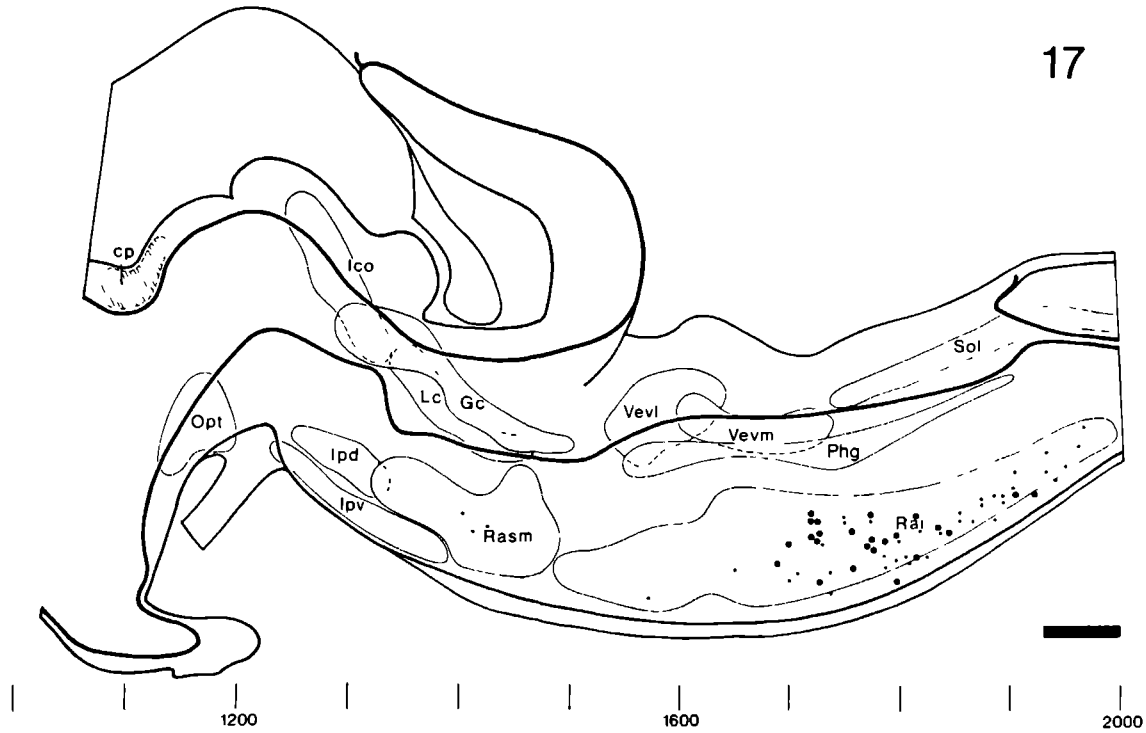
The *nucleus reticularis inferior* (Figs 13-16, 17, 22) as distinguished in the present study is comparable to the nucleus reticularis inferior pars dorsalis (RID) as defined by Newman and Cruce (1982) In the rostral half of the nucleus reticularis inferior in *Varanus exanthematicus* the number of large cells is much higher than in its caudal half or in the nucleus reticularis medius, here the delineation from the nucleus raphes inferior is not distinct In the caudal half, very large cells are almost completely lacking, except for the region caudal to the obex Whereas the nucleus reticularis superior and nucleus reticularis medius send their spinal projections via the flm into the ventral funiculus of the spinal cord, the nucleus reticularis inferior projects mainly via the lateral funiculus (Robinson 1969, ten Donkelaar 1976a, Cruce and Newman 1981, Wolters et al 1982, ten Donkelaar and de Boer-van Huizen 1984a)

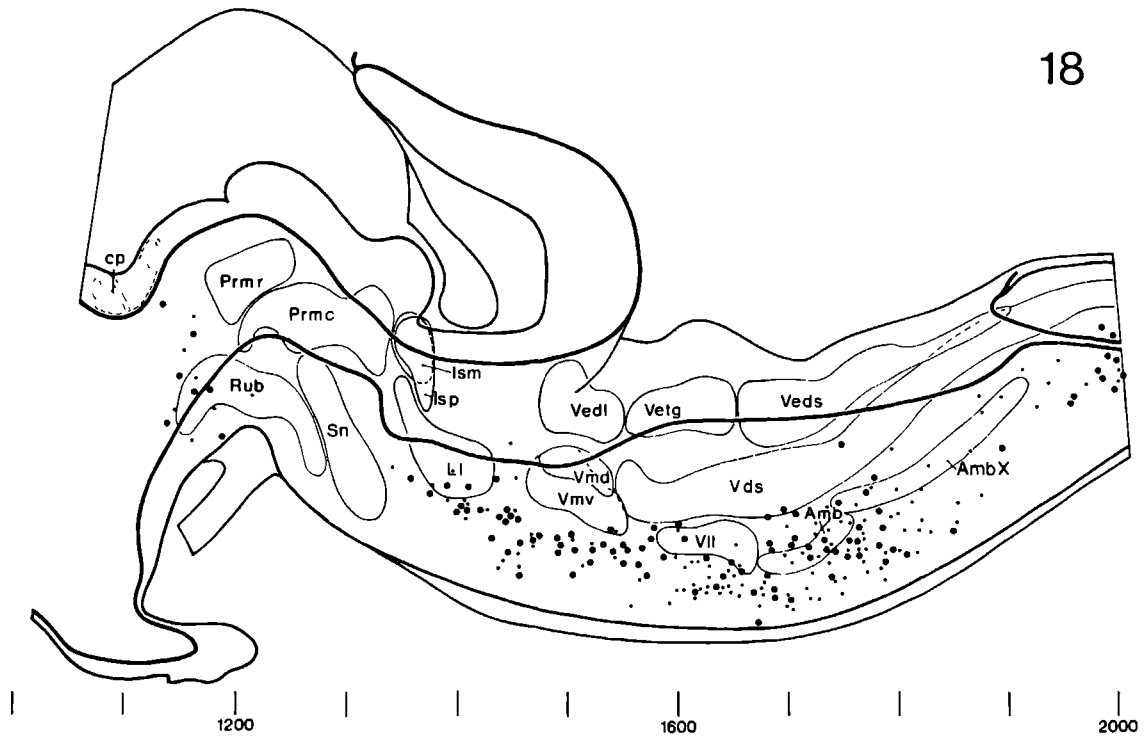
The *nucleus reticularis inferior pars ventralis* (Figs 13, 14, 17, 22) is a small ventral cluster of closely packed, mainly large and medium-sized cells, adjacent to the nucleus raphes inferior and the lemniscus medialis It accompanies the rostral half of the nucleus reticularis inferior, and fades at the level where the nucleus tractus solitarii and the nucleus vestibularis ventromedialis merge This subnucleus shows a distinct bilateral spinal projection with a conspicuously high degree of collateralization (Wolters et al 1985a)

Along the caudal one-third of the nucleus reticularis inferior, adjacent to the nucleus raphes inferior and the lemniscus medialis, a distinct *inferior olive* (Figs 15, 16) was found after HRP injections into the cerebellum (Bangma and ten Donkelaar 1982, Kunzle and Wiklund 1982, Kunzle 1983, 1985) This nucleus consists of loosely arranged medium-sized cells, and cannot easily be delineated in normal material

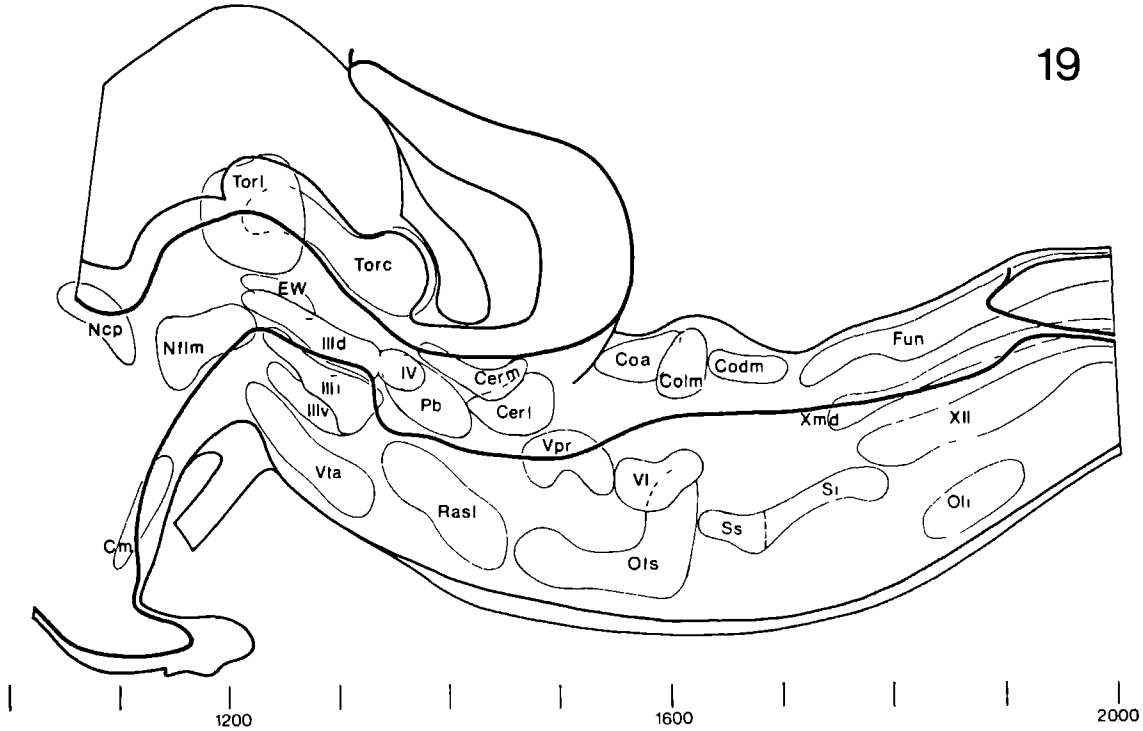
In the lateral reticular zone two subnuclei have been recognized in retrograde tracer studies (Wolters et al 1985a). Adjacent to the nucleus reticularis medius the *nucleus reticularis medius pars lateralis* is found (Figs 10-12), a distinct cluster of small to medium-sized cells, just ventral to the nucleus descendens nervi trigemini Possibly the nucleus reticularis medius pars lateralis corresponds to the nucleo metencefalico inferiore laterale of Stefanelli (1944)

After retrograde tracer experiments ("Fast Blue" and "Nuclear Yellow" application to the spinal cord), also a lateral cell cluster is found labeled which extends throughout the caudal rhombencephalon and takes a dorsolateral position more caudally This cell cluster is called the *nucleus reticularis inferior pars lateralis* (Figs 13-16) At the level of the obex this nucleus is most distinct, whereas the number of labeled cells is gradually





Figs. 17-19. Topographical reconstructions of the cell masses in the brain stem and caudal diencephalon of the lizard *Varanus exanthematicus*, as projected upon a sagittal plane. Dots in Figs. 17 and 18 represent one out of five large and very large cell bodies in the medial magnocellular reticular zone and in the raphe nuclei, respectively. In order to obtain some three-dimensional insight, the outlines of more laterally situated cell masses which project within the confines of medial nuclei have been indicated with thin broken lines. The scales along the figures indicate section numbers and correspond with the numbers at the right side of the Figs. 3-16. (Bar: 1 mm)



decreasing rostrally. Possibly the nucleus reticularis inferior pars lateralis corresponds to both the nucleo mielencefalico superiore laterale and the nucleo mielencefalico principale laterale of Stefanelli (1944). From both nucleus reticularis medius pars lateralis and nucleus reticularis inferior pars lateralis ascending as well as descending projections have been established (ten Donkelaar and de Boer-van Huizen 1984b; Wolters et al. 1985a).

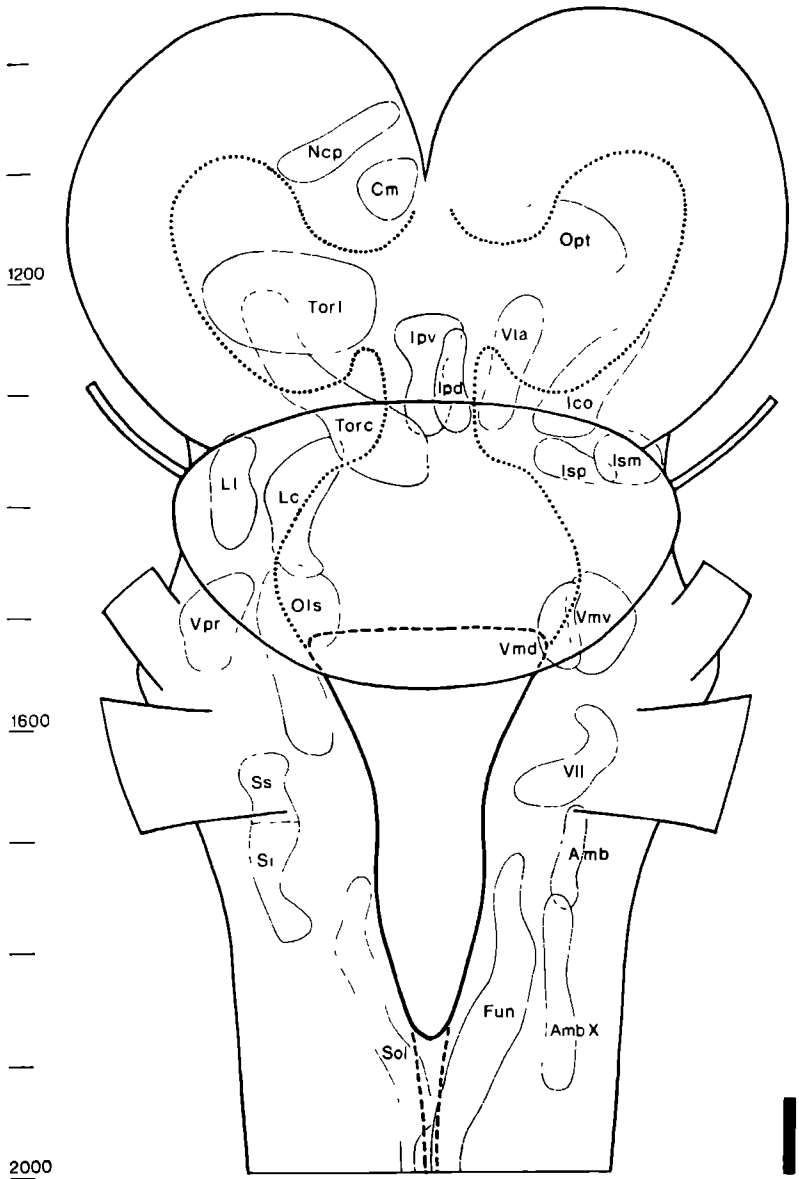
Nuclei of the isthmic region

Under this heading the following cell masses will be briefly considered: the nucleus isthmi, the locus coeruleus, the parabrachial region, the central grey, and the interpeduncular nucleus.

The *nucleus isthmi pars magnocellularis* (Fig. 7) is a conspicuous cell mass directly adjacent to the dorsolateral brain stem surface of the rostral isthmic region. It consists of densely packed medium-sized cells Medially, this nucleus is bordered by the *nucleus isthmi pars parvocellularis* (Fig. 7), which contains compactly arranged small and very small cells. Its rostral delineation from the nucleus profundus mesencephali pars caudalis, at the level of the caudal pole of the tectum mesencephali, is difficult; caudally this nucleus borders on the cerebellar nuclei and caudoventrally on the parabrachial region. Although the functional significance of both parts of the nucleus isthmi remains obscure, experimental data in various reptiles showed reciprocal connections with the tectum mesencephali (Foster and Hall 1975; ten Donkelaar 1976b, ten Donkelaar and de Boer-van Huizen 1981b; Kunzle and Schnyder 1984), and suggest a visual function. From the magnocellular part of the nucleus isthmi also a projection to the habenula was established (Hoogland 1982).

As *parabrachial region* (Figs. 7, 8) the fairly well delimited cell mass is entitled which surrounds the brachium conjunctivum, and consists of very small and small cells. Rostrally the parabrachial region extends ventral to the nucleus isthmi pars parvocellularis, caudally it is continuous with the lateral cerebellar nucleus. Most probably the parabrachial region includes the nucleus visceralis secundarius as distinguished by Shanklin (1930) and Barnard (1936), Cruce and Nieuwenhuys (1974), and ten Donkelaar and Nieuwenhuys (1979). The parabrachial region has extensive ascending projections beyond the mesencephalon (ten Donkelaar and de Boer-van Huizen 1981b, 1984b), and receives input from the nucleus tractus solitarii (ten Donkelaar and de Boer-van Huizen 1981b). Moreover the extensive peptidergic (substance P, [Leu]- and [Met]enkephalin) innervation of this region (Wolters et al. 1985c) indicates that the parabrachial region probably forms a relay nucleus in projections related to visceral and taste information.

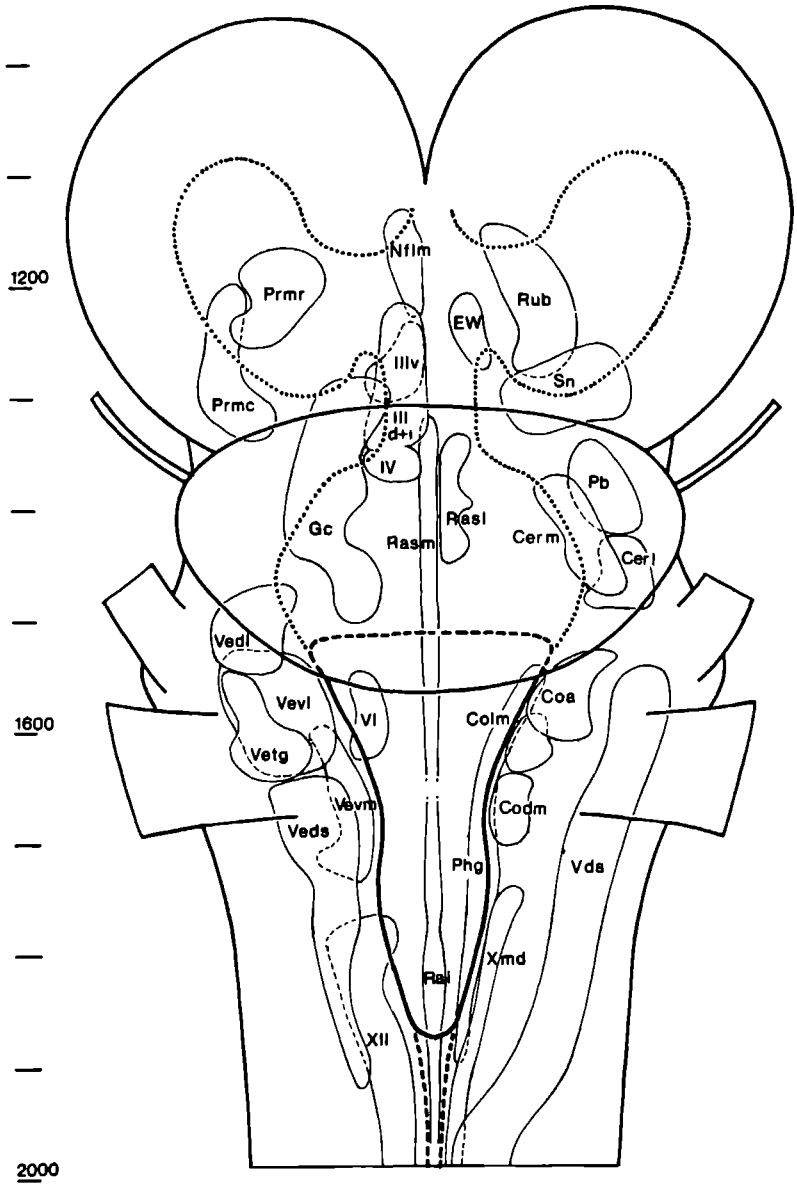
The *nucleus lemnisci lateralis* (Figs. 7-9) consists of very small to medium-sized cells, and lies in the lateral isthmic region, ventral to the parabrachial



Figs. 20-22. Topographical reconstructions of the cell masses in the brain stem and caudal diencephalon of the lizard *Varanus exanthematicus*, as projected upon a horizontal plane. In order to obtain some three-dimensional insight, the outlines of more ventrally situated cell masses which project within the confines of dorsal nuclei have been indicated with thin broken lines. Dotted lines in Figs. 20-21 indicate the extent of the ventricle at the mesencephalic and the rostral rhombencephalic levels. Dots in Figs. 22 represent one out of five large and very large cell bodies in the medial magnocellular reticular zone and in the raphe nuclei. The various reticular nuclei, as distinguished in the present study, have been indicated schematically with thin broken lines. The scales along the figures indicate section numbers and correspond with the numbers at the right side of the Figs. 3-16. (Bar: 1 mm)

region and the nucleus cerebellaris lateralis, medial to the fibers of the lateral lemniscus.

The *locus coeruleus* (Figs. 7-9) consists of scattered medium-sized cells medial to the parabrachial region and the nucleus lemnisci lateralis, but dorsal to the large elements of the nucleus reticularis superior and nucleus reticularis superior pars lateralis. This poorly delimited cell mass could easily be delineated in an immunohistochemical study, using anti-tyrosine-hydroxylase (Wolters et al. 1984). In addition to the catecholaminergic locus coeruleus cells, a group of scattered cells ventrolateral to the locus coeruleus was labeled in these experiments, constituting the *locus subcoeruleus* (Figs. 7-9). This cell group, however, cannot be distinguished in normal material. The locus coeruleus and subcoeruleus



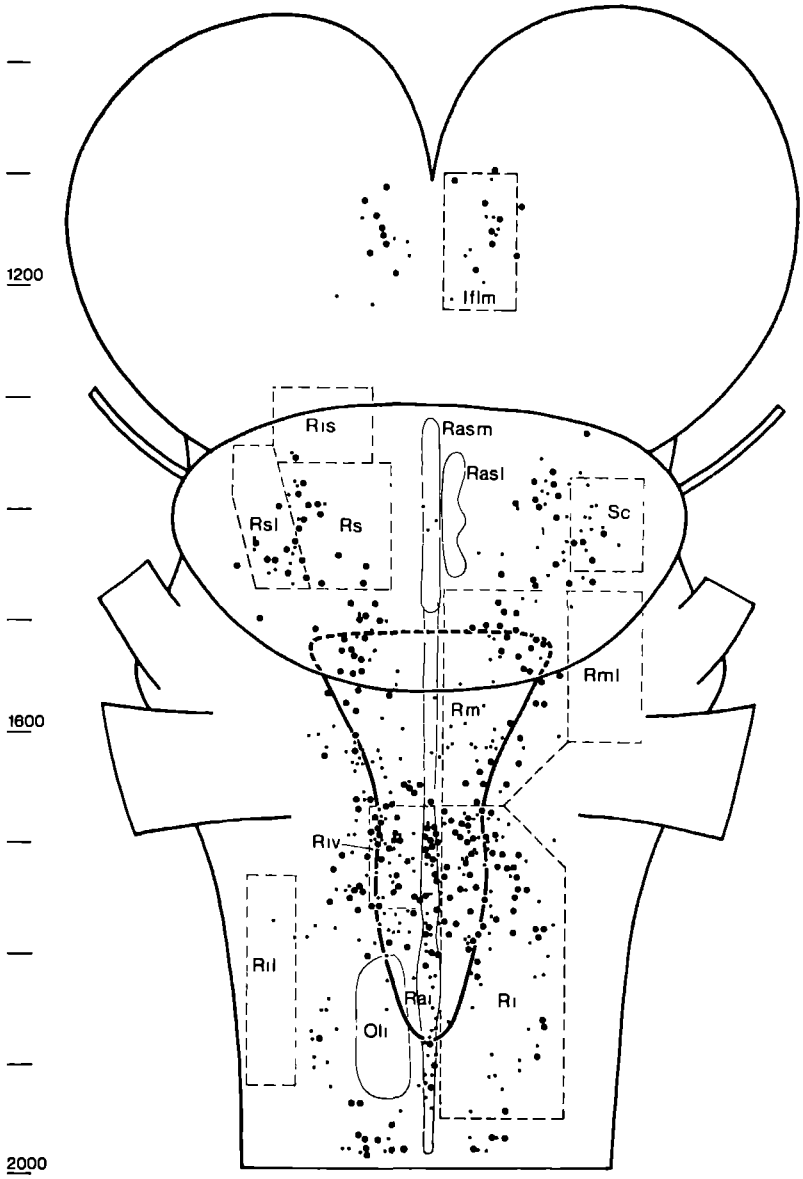
have extensive spinal projections (ten Donkelaar et al. 1980; ten Donkelaar 1982; Woodson and Künzle 1982; Newman et al. 1983; Wolters et al. 1982, 1985a).

The *griseum centrale* (Figs. 6-10) is a narrow zone of very small and small cells, forming a periventricular layer from the caudal isthmic level up to the caudal mesencephalic level, where it enlarges, and takes a dorsolateral position to the aqueductus cerebri. Here catecholaminergic (Wolters et al. 1984) and enkephalinergic (Wolters et al. 1985c) neurons have been found, as well as a dense enkephalinergic innervation (Wolters et al. 1985c).

The interpeduncular nucleus, which receives the fasciculus retroflexus (see Distel and Ebbesson 1981), can be divided into a dorsal and a ventral part.

The *nucleus interpeduncularis pars ventralis* (Figs. 6-8) is an unpaired elongated nucleus ventral to the nucleus raphes superior, and consists of very small cells. It extends from the caudal mesencephalic to the middle isthmic level. Substance P- and [Leu]-enkephalin-containing cell bodies were found in the nucleus interpeduncularis pars ventralis, as well as a serotonergic (Wolters et al. 1985b) and peptidergic (Wolters et al. 1985c) innervation.

The small-celled *nucleus interpeduncularis pars dorsalis* (Fig. 6) extends rostrally between the oculomotor nerve roots and the nucleus raphes superior pars lateralis, with which it is directly continuous. It contains some catecholaminergic neurons



(Wolters et al. 1984).

Mesencephalic nuclei

The most prominent part of the reptilian mesencephalon is the *tectum mesencephali* (Figs. 3-6), which receives the bulk of the fibers from the optic nerve via the crossed and, for a minor part, via the uncrossed optic tract. This coarse and compact fiber bundle innervates the superficial layers of the tectum. Although several authors distinguish fourteen layers in the reptilian tectum mesencephali (Ramón 1896; Senn 1968; Butler and Northcutt 1971; Butler and Ebbesson 1975; Northcutt 1984), the present account follows the division into six layers as made by Huber and Crosby (1926, 1933) and ten Donkelaar and Nieuwenhuys (1979). They distinguish, from superficial to deep: 1. the *stratum opticum*, which receives the fibers from the optic tract; 2. the *stratum fibrosum et griseum superficiale*, consisting of loosely arranged, laminarly organized small and very small cells; 3. the *stratum griseum centrale*, a thick layer of darkly stained, densely packed very small to medium-sized cells; 4. the *stratum album centrale*, in which predominantly the axons of the cells in the former layer descend; 5. the *stratum griseum periventriculare*, a narrow layer of loosely arranged small and medium-sized neurons; 6. the *stratum fibrosum periventriculare*, the innermost fiber layer.

Efferent ascending (tectothalamic) and descen-

ding (tectobulbar) projections from the tectum mesencephali in several reptiles have been demonstrated experimentally (see Northcutt 1984 for review).

In the tegmentum mesencephali various nuclei can be distinguished. In the rostral part: nucleus opticus tegmenti, nucleus of the flm, nucleus laminaris of the torus semicircularis, nucleus profundus mesencephali pars rostralis and nucleus ruber; in the caudal part: nucleus centralis of the torus semicircularis, nucleus profundus mesencephali pars caudalis, nucleus intercollicularis, and substantia nigra.

The *nucleus opticus tegmenti* (Fig. 4), or *nucleus of the basal optic root*, is found in the rostral mesencephalic tegmentum, at the ventrolateral surface of the brain stem. It consists of loosely arranged medium-sized cells, and forms the projection area of the basal optic root. From this nucleus, an extensive ipsilateral projection to the cerebellum was demonstrated (Bangma and ten Donkelaar 1982; Künzle 1983), as well as ipsilateral descending projections to the rhombencephalon (ten Donkelaar and de Boer-van Huizen 1981a).

The *nucleus of the flm* (Figs. 4, 5) is a slender nucleus medial to the flm in the rostral part of the mesencephalon. It is composed of densely packed, small spindle-shaped neurons, and gives rise to a predominantly ipsilateral spinal projection (ten Donkelaar et al. 1980; Wolters et al. 1985a).

The *nucleus laminaris of the torus semicircularis* (Figs. 4, 5) forms a flattened periventricular cell group, ventral to the lateral recess of the third ventricle. It consists of tightly packed medium-sized and small cells. It is directly continuous, medially, with the nucleus of the flm, and, rostrally, with the nucleus periventricularis hypothalami, from which it cannot easily be delimited. Laterally it merges with the stratum griseum periventriculare of the tectum mesencephali. The nucleus laminaris of the torus semicircularis receives spinal afferents (Ebbesson 1967, 1969) and has a projection to the spinal cord (ten Donkelaar and de Boer-van Huizen 1978, 1984a; ten Donkelaar et al. 1980; Butler and Bruce 1981; Woodson and Künzle 1982).

The *nucleus centralis of the torus semicircularis* (Figs. 4-7) consists of diffusely arranged small and medium-sized cells. It extends from the lateral part of the nucleus laminaris of the torus semicircularis, by which it is roofed, in mediocaudal direction, to the level of the velum medullare anterius, where it forms a bilateral dorsal protrusion. The reptilian nucleus centralis of the torus semicircularis is generally considered homologous to the mammalian inferior colliculus. It receives the secondary acoustic projections (Hartline 1971; Foster and Hall 1978; Kennedy 1975) via the lateral lemniscus, and gives rise to ascending projections (Foster and Hall 1978; Pritz 1974; Hoogland 1982).

In the central part of the tegmentum mesencephali the nucleus profundus mesencephali is found, which consists of a rostral and a caudal part.

The *nucleus profundus mesencephali pars rostralis* (Figs. 4, 5) is a diffuse cell mass throughout the center of the mesencephalic tegmentum, consisting of small and medium-sized cells.

The *nucleus profundus mesencephali pars caudalis* (Figs. 5, 6) lies ventrally to the caudal part of the former nucleus, and consists of small cells with some scattered medium-sized elements. It extends further caudally, where the borders with the nucleus isthmi pars parvocellularis and the nucleus intercollicularis are indistinct. The functional significance of both parts of the nucleus profundus mesencephali is still unknown. A dense serotonergic innervation of the nucleus profundus mesencephali pars rostralis was demonstrated (Braak et al. 1968; Wolters et al. 1985b).

The *nucleus intercollicularis* (Fig. 6) is a diffuse cell mass in the laterodorsal part of the caudal mesencephalon, consisting of mainly small cells. Its boundaries, laterally with the nucleus profundus mesencephali pars caudalis, and medially with the griseum centrale, are indistinct.

The *nucleus ruber* (Figs. 4, 5) consists mainly of diffusely arranged medium-sized cells throughout the ventral part of the mesencephalic tegmentum. The boundaries with the substantia nigra and the ventral tegmental area, are indistinct. An extensive contralateral spinal projection arises from the nucleus ruber (Robinson 1969; ten Donkelaar et al. 1980; Wolters et al. 1982, 1985a), as well as a modest projection to the lateral cerebellar nucleus (Bangma and ten Donkelaar 1982; ten Donkelaar and Bangma 1985).

The *substantia nigra* (Fig. 6) is a large diffuse cell mass in the ventrolateral part of the caudal mesencephalic tegmentum, consisting of very small to medium-sized elements. It cannot easily be delineated in normal material; only a certain condensation of neurons indicates its localization. Immunohistochemical (Wolters et al. 1984) as well as histofluorescence studies (Parent 1979; Brauth and Kitt 1980; Brauth et al. 1983) revealed the presence of catecholaminergic cell bodies in this region. In *Varanus exanthematicus* these neurons are located in the dorsal part of, and dorsal to the area which could be identified as substantia nigra in normal material. In particular the ventral part of this area receives a very dense substance P innervation (Brauth et al. 1983; Reiner et al. 1984; Wolters et al. 1985c). Thus evidence has been gathered for the existence of two parts of the substantia nigra, which may be considered homologous to the mammalian *pars compacta* and *pars reticulata*, respectively.

The *ventral tegmental area* (Figs. 5, 6) is a small-celled region ventromedial to the substantia nigra, which extends from this level more rostrally, where its cells are intercalated among the fibers of the oculomotor nerve root. It cannot easily be delineated in normal material, but its extent was determined in several immunohistochemical studies,

in which a great many catecholaminergic (Brauth et al 1983, Wolters et al 1984) and fewer serotonergic (Brauth et al 1983, Wolters et al 1985b) cell bodies could be demonstrated in this area This region receives a dense substance P (Brauth et al 1983, Reiner et al 1984, Wolters et al 1985c) and serotonin (Brauth et al 1983, Wolters et al 1985b) innervation From the ventral tegmental area ascending projections to the forebrain were established (Lohman and van Woerden-Verkley 1978, Brauth and Kitt 1980, ten Donkelaar and de Boer van Huizen 1981b) Thus several arguments have been gathered which show the correspondence between the reptilian and the mammalian ventral tegmental area

Hypothalamic nuclei

In this paragraph only those nuclei in the caudal diencephalon will be briefly considered which give rise to descending spinal projections, or which were conspicuously labeled in our immunohistochemical studies (Wolters et al 1984, 1985b, 1985c) For a review of the cell masses in a lizard diencephalon we refer to Cruce (1974), whose nomenclature has been adopted for the greater part in the present account

The *nucleus periventricularis hypothalami* (Fig 3) forms a distinct vertical periventricular cell layer along the narrowest part of the third ventricle It consists of small and very small cells, with some scattered medium-sized elements Caudally it merges with the nucleus laminaris of the torus semicircularis and the nucleus of the flm The nucleus periventricularis hypothalami gives rise to a modest, but highly collateralizing, predominantly ipsilateral spinal projection (ten Donkelaar et al 1980, Woodson and Kunzle 1982, Wolters et al 1985a) Ascending projections were found to various thalamic nuclei (Hoogland 1982) In the nucleus periventricularis hypothalami many substance P-containing (Brauth et al 1983, Reiner et al 1984, Wolters et al 1985c) and [Leu]- and [Met]enkephalinergic (Wolters et al 1985c) neurons were demonstrated, as well as a distinct substance P innervation (Brauth et al 1983, Reiner et al 1984) A rather dense enkephalinergic innervation of the dorsal part of this nucleus was found (Wolters et al 1985c), and a modest serotonergic innervation of its ventral part (Wolters et al 1985b)

Medial to the middle part of the former nucleus the small but distinct *paraventricular organ* (Fig 3) is found, which consists of tightly packed small bipolar cells They constitute a highly vascularized specialization of the ependymal layer, delineating the sulcus lateralis infundibuli These neurons are in direct contact with the cerebrospinal fluid of the third ventricle by means of a short club-like process They have been shown to contain serotonin (Braak et al 1968, Ueda et al 1983, Wolters et al 1985b) and catecholamines (Baumgarten and Braak 1968, Parent and Poirier 1971, Parent and Poitras 1974)

The *nucleus ventralis hypothalami* (Fig 3) is a conspicuous cell mass in the most ventral part of the hypothalamus, which consists mainly of very small and small cells, with some scattered medium-sized elements It contains large amounts of [Leu] and [Met]enkephalinergic neurons, and its ventrolateral part receives a dense enkephalinergic innervation (Wolters et al 1985c)

The *area lateralis hypothalami* (Fig 3) is a poorly defined area dorsal to the nucleus ventralis hypothalami and lateral to the nucleus periventricularis hypothalami, which consists of diffusely arranged small and medium sized neurons Many catecholaminergic neurons are found in its central part (Wolters et al 1984), substance P containing neurons in its dorsal part (Wolters et al 1985c) and enkephalinergic neurons in its ventral part, continuous with the enkephalinergic cell cluster in the nucleus ventralis hypothalami (Wolters et al 1985c) The entire area lateralis hypothalami shows a very dense serotonergic (Wolters et al 1985b) innervation, as well as a dense enkephalinergic innervation of its ventrolateral part (Wolters et al 1985c) An extensive ipsilateral spinal projection, especially to cervical and high thoracic levels, has been demonstrated (ten Donkelaar et al 1980, Wolters et al 1985a)

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FUNICULAR TRAJECTORIES OF DESCENDING BRAIN STEM PATHWAYS IN A LIZARD, VARANUS EXANTHEMATICUS

INTRODUCTION

Many authors have studied the descending pathways from the brain stem to the spinal cord with the horseradish peroxidase (HRP) technique in mammals (e.g., Kuypers and Maisky, 1975; Crutcher et al., 1978; Kneisley et al., 1978; Martin et al., 1979) as well as in non-mammalian vertebrates (e.g., Ten Donkelaar and de Boer-van Huizen, 1978; Newman and Conte, 1980; Ten Donkelaar et al., 1980, 1981; Cruce et al., 1981; Smeets and Timerick, 1981). Attention was paid also to the funicular trajectories of the various pathways demonstrated, taking advantage of the fact that damaged axons can take up HRP and transport this enzyme to their parent cell bodies (Kristensson and Olsson, 1974; Kuypers and Maisky, 1975). However, no conclusive evidence could be obtained on the precise localization of many descending pathways within the various spinal funiculi. Therefore, HRP injections were combined with large spinal lesions so as to allow HRP transport through only a restricted part of the spinal white matter (Kuypers and Maisky, 1977; Martin et al., 1978, 1979, 1981; Basbaum and Fields, 1979) or HRP was injected into various parts of the spinal funiculi (Tohyama et al., 1979). Recently, another technique has become available making use of HRP slow-release gels (Griffin et al., 1979; Watkins et al., 1980). In this way the application of HRP can be restricted to a particular part of a spinal funiculus and a major difficulty with the use of HRP, viz., its spread in neural tissue, could in part be overcome.

In the present study HRP slow-release gels have been applied to various parts of the lateral and ventral funiculi of the cervical enlargement in the lizard *Varanus exanthematicus* in order to obtain more conclusive evidence on funicular trajectories of various descending pathways. Previous data on the cells of origin of descending pathways and their funicular trajectories have been reviewed by Ten Donkelaar (this volume).

MATERIALS AND TECHNIQUES

In the present study 8 lizards *(Varanus exanthematicus)* were used, varying in weight from 200 to 650 g, with a total length of 50–70 cm and a snout-vent length of 25–35 cm. All animals were intubated and received endotracheal anesthesia for which a mixture of oxygen, nitrous oxide with 1/4–1/2 volumen % halothane was used. Under aseptic conditions a midline skin

incision was made, the dorsal musculature was bilaterally separated and a laminectomy was performed with the aid of a Zeiss binocular operation microscope. After incision of the dura, the HRP slow-release gel was implanted with a fine-tipped forceps into the desired quadrant of the spinal cord.

The preparation of the HRP slow-release gel (Griffin et al., 1979) was slightly modified to obtain a slower release of the enzyme and a less fragile dried gel, which would be easier to handle and to apply. Therefore, the concentrations of acrylamide and N,N'-methylene-bis-acrylamide used were 75% instead of 44.4% and 15% instead of 1.2% w/v, respectively.

After the operation the animals survived for 7 days at an environmental temperature of 25–28°C, and were then perfused transcardially under deep Nembutal anesthesia with a mixture of 1% formaldehyde and 1.25% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). The brain and spinal cord were removed, stored at 4°C in phosphate buffer containing 30% sucrose during 5 h and afterwards embedded in gelatine (15% gelatine and 30% sucrose in phosphate buffer). This was stored at 4°C overnight in 10% formaldehyde, and after being placed in 30% sucrose-phosphate buffer for 2 h the gelatine block containing brain and spinal cord was cut on a freezing microtome into sections of 40 μm in the transversal plane. The sections were incubated in a medium containing tetramethylbenzidine (TMB) and hydrogen peroxide, according to a slightly modified Mesulam (1978) procedure (Ten Donkelaar et al., 1980) and mounted in Depex. For optimal visualization of the implantation site, the diaminobenzidine (DAB) procedure according to Graham and Karnovsky (1966) was used in a parallel series of sections.

RESULTS

(a) Pathways descending via the dorsal part of the lateral funiculus

Following implantation of HRP slow-release gel in the dorsal part of the lateral funiculus (e.g. case 6196, Fig. 1) many labeled neurons were observed throughout the brain stem. No labeled neurons were found in the hypothalamus. A few labeled cells were present in the ipsilateral interstitial nucleus of the fasciculus longitudinalis medialis (flm). As expected, the contralateral red nucleus (see Ten Donkelaar, this volume, Fig. 3) contained many retrogradely labeled cells and most of them were located in its medial part (Fig. 1A). At the isthmic level, labeled cells were observed in the locus coeruleus (Fig. 1B, C) and in two reticular cell groups. Ipsilaterally, the subcoeruleus area contained a large number of labeled neurons (Fig. 1C), contralaterally a distinct group of labeled medium-sized neurons was found (Fig. 1C, D). The latter cell group was already noted in previous HRP studies (Ten Donkelaar and de Boer-van Huizen, 1978; Ten Donkelaar et al., 1980) and probably is comparable to the lateral pontine area observed in mammals (Kuypers and Maisky, 1975; Hancock and Fougerousse, 1976; Martin et al., 1979; Tohyama et al., 1979).

No labeled cells were observed in the nucleus reticularis superior, i.e., the rostral group of the large-celled medial reticular zone (see Ariëns Kappers et al., 1936; Ten Donkelaar and Nieuwenhuys, 1979, for reviews), and only a few in the nucleus reticularis medius, mainly contralaterally (Fig. 1E). Many labeled cells were present throughout the nucleus reticularis inferior (Fig. 1F–J), especially ipsilaterally; at caudal levels a lateral shifting of the labeled cells was observed (Fig. 1I, J). In the nucleus raphe inferior labeled neurons were present only in a restricted area (Fig. 1H). Finally, a conspicuous group of labeled cells was observed in and around the dorsal motor nucleus of the vagus nerve (Fig. 1H), and furthermore labeled cells

were present in the nucleus of the solitary tract, in its rostral part contralaterally (Fig. 1H), in its caudal part ipsilaterally (Fig. 1I), in keeping with observations by Ten Donkelaar et al. (1980).

(b) Pathways descending via more ventral parts of the lateral funiculus

The HRP gel implantation in the lateral funiculus in case 6210 (Fig. 2) remained restricted to a small, ventral area and forms an illustration of the advantage of a rather limited localization of HRP using slow-release gels. In such cases, only a modest number of cells were labeled throughout the brain stem. In the hypothalamus labeled cells were observed, viz., in the nucleus paraventricularis (Fig. 2A). No labeled cells were observed in the mesencephalon. In the rostral part of the rhombencephalon a few labeled neurons were found ipsilaterally in the locus coeruleus (Fig. 2B,C), contralaterally in the reptilian homologue of the lateral pontine area (Fig. 2B) and in the medial cerebellar nucleus (Fig. 2C). A few labeled cells were also noted in the nucleus reticularis medius (Fig. 2D–F), in the nucleus reticularis inferior (Fig. 2 G–J), in the nucleus raphe inferior (Fig. 2H) and in the nucleus of the solitary tract (Fig. 2I).

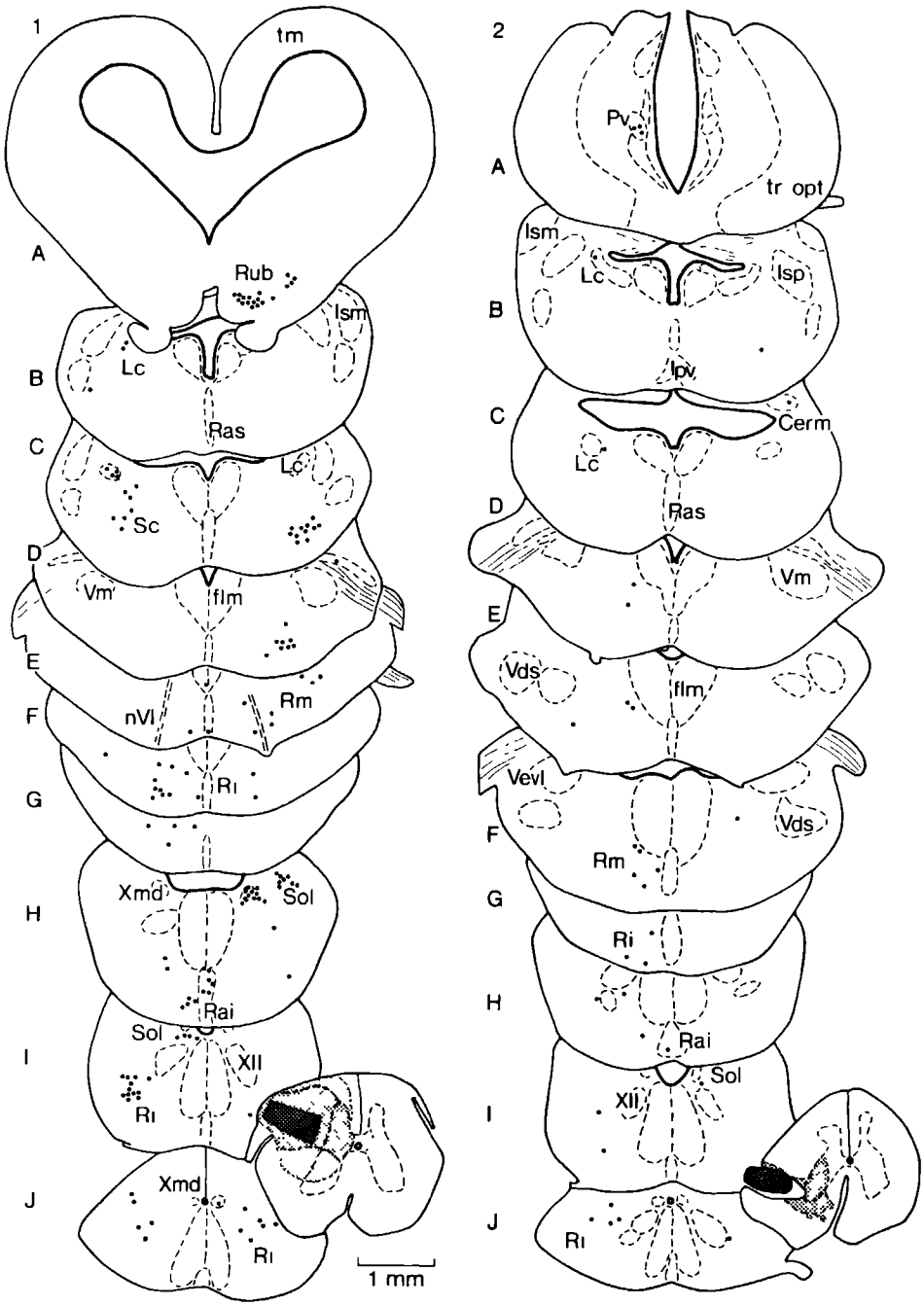
(c) Pathways descending by way of the ventral funiculus

As illustrated in Fig. 3 after the HRP gel implantation in the ventral funiculus many labeled cells were observed in various brain stem areas, some of which were already known to project via that part of the spinal white matter (see Ten Donkelaar, this volume). No labeled cells were observed in the hypothalamus. In the mesencephalon only a few labeled neurons were found in the interstitial nucleus of the flm. In the most rostral part of the rhombencephalon (Fig. 3A, B) many labeled cells were observed in the ipsilateral subcoeruleus area (Fig. 3A), in the contralateral medial cerebellar nucleus (Fig. 3A), but also in the rostral part of the rhombencephalic magnocellular reticular formation, i.e., the nucleus reticularis superior (Fig. 3B). Two distinct groups of labeled cells were observed in the ipsilateral nucleus reticularis medius (Fig. 3C, E). In keeping with previous data (e.g., Ten Donkelaar et al., 1980) a large number of labeled neurons were observed in the nucleus vestibularis ventrolateralis (Fig. 3D, E), i.e., the reptilian homologue of the mammalian nucleus of Deiters. Throughout the nucleus reticularis inferior labeled cells were found, but also in the nucleus raphe inferior, particularly in its caudal part (Fig. 3F–H).

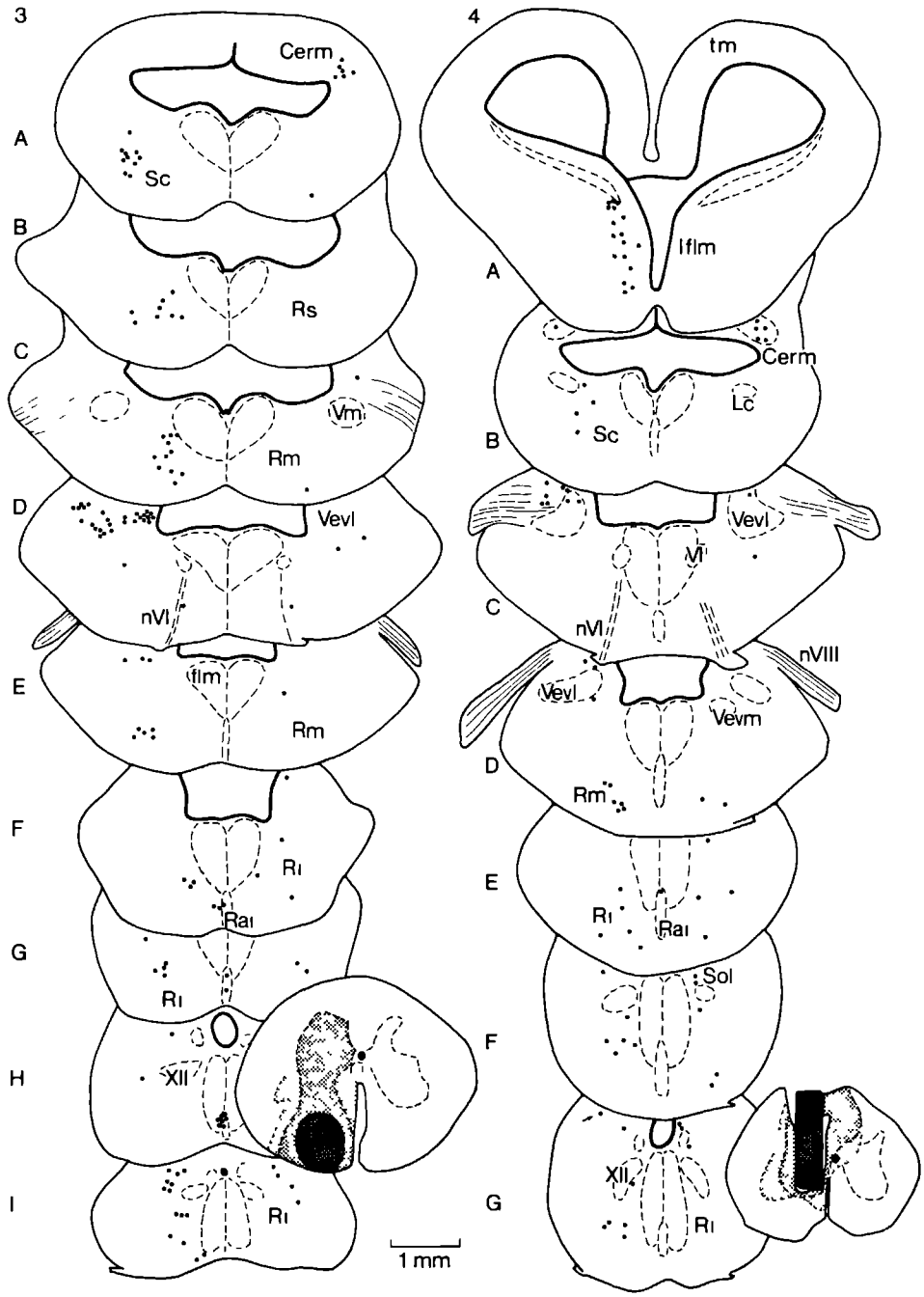
(d) Descending pathways via the dorsalmost part of the ventral funiculus

Peculiar to lizards, the most dorsal part of the ventral funiculus is separated from the remainder by an accessory commissure (Kusuma et al., 1979). This dorsal portion of the ventral funiculus was found to contain interstitiospinal fibers, arising in the interstitial nucleus of the flm as shown in the lizard *Tupinambis nigropunctatus* (Ten Donkelaar, 1976b). To reach this particular area in the spinal cord a gel was implanted via the dorsal funiculus. The gel also infiltrated the most dorsal part of the remainder of the ventral funiculus. In the dorsal funiculus descending fibers are very few (see Ten Donkelaar, this volume), and probably arise in the dorsal column nuclei (see Fig. 4G, arrow).

As expected most labeled cells were found in the ipsilateral interstitial nucleus of the flm (Fig. 4A). Some ipsilateral subcoeruleus cells were also labeled (Fig. 4B) and labeled neurons were observed in the medial cerebellar nucleus mainly contralaterally (Fig. 4B). In the vestibular nuclear complex labeled cells were observed in the dorsal part of the nucleus vestibularis ventrolateralis (Fig. 4C, D). In the reticular formation labeled cells were found in



Figs. 1 and 2. HRP slow-release gel implantations in the lateral funiculus at the seventh spinal segment of the lizard *Varanus exanthematicus* Dark gray stained is the HRP slow-release gel, the lighter area around the gel represents the HRP in the damaged neural tissue, while the spread of the enzyme beyond the implantation site is indicated by a light gray color.



Figs 3 and 4 HRP slow-release gel implantations in the ventral funiculus at the seventh spinal segment of the lizard *Varanus exanthematicus* For code see Figs. 1 and 2

the caudal part of the nucleus reticularis medius (Fig 4D) and scattered throughout the nucleus reticularis inferior (Figs 4E–G), particularly ipsilaterally A few labeled cells were observed in the nucleus raphes inferior (Fig 4E) and in the nucleus of the solitary tract (Fig 4F–G)

DISCUSSION

In the present study HRP slow-release gels were placed in various parts of the spinal white matter, thereby lesioning fibers only locally Damaged fibers are known to take up HRP and transport the enzyme to their parent cell bodies (Kristensson and Olsson, 1974, Kuypers and Maisky, 1975, Ten Donkelaar and de Boer-van Huizen, 1978, Ten Donkelaar et al 1980) It may be assumed that most of the retrograde labeling in the brain stem resulted from incorporation of HRP by axons injured by the implant However, since some spread of HRP in the gray matter (see Figs 1–4) could not be avoided, some of the neuronal labeling may be due to uptake of the enzyme by terminal structures The presence of a few labeled neurons in e g the interstitial nucleus of the flm after a slow-release gel in the dorsal part of the lateral funiculus can be explained in this way, since this nucleus evidently projects via the dorsalmost part of the ventral funiculus (Ten Donkelaar, 1976b, the present study)

In the present study previous HRP data (Ten Donkelaar and de Boer-van Huizen, 1978, Newman and Conte, 1980, Ten Donkelaar et al , 1980, Cruce et al , 1981) have been confirmed, but in addition the funicular trajectories of various descending pathways could be demonstrated In keeping with previous data the hypothalamic paraventricular nucleus was shown to project via the lateral funiculus, the interstitial nucleus of the flm via the dorsalmost part of the ventral funiculus and the nucleus ruber by way of the dorsal part of the lateral funiculus

The projections from the locus coeruleus and the subcoeruleus area have a wider funicular distribution than had been assumed on the basis of earlier observations (Ten Donkelaar et al , 1980) In addition to ipsilateral projections via the dorsal part of the lateral funiculus, such fibers pass by way of the remainder of the lateral funiculus but also via the ventral funiculus These findings are in agreement with data in mammals (Kuypers and Maisky, 1977, Nygren and Olson, 1977, Basbaum and Fields, 1979, Martin et al , 1979, Westlund and Coulter, 1980) The funicular trajectory of the fibers from the cell group presumably comparable to the mammalian lateral pontine area is restricted to the dorsal part of the lateral funiculus which is in keeping with data in mammals (Kuypers and Maisky, 1977, Martin et al , 1979) This area has already been recognized in reptiles by Stefanelli (1944) as the superior lateral metencephalic nucleus

The funicular trajectories of projections arising in the magnocellular reticular formation are largely in agreement with earlier findings (see Ten Donkelaar, this volume) Projections from the nucleus reticularis superior and the nucleus reticularis medius were found situated in the ventral funiculus In addition, a contralateral projection from the nucleus reticularis medius via the dorsal part of the lateral funiculus has been demonstrated (Fig 1E) Reticulospinal fibers arising in the nucleus reticularis inferior, however, have a definitely wider funicular distribution than previously known In keeping with earlier findings in reptiles (Ten Donkelaar, 1976a,b, Ten Donkelaar et al , 1980, Cruce and Newman, 1981), a spinal projection via the lateral funiculus including its dorsal part has been demonstrated (Figs 1F and 2G) A distinct reticulospinal projection arising in the nucleus reticularis inferior, however, was found distributed through the entire ventral funiculus (Figs 3F and 4E) This wider funicular distribution of reticulospinal fibers is in keeping with data in mammals (e g , Kuypers and

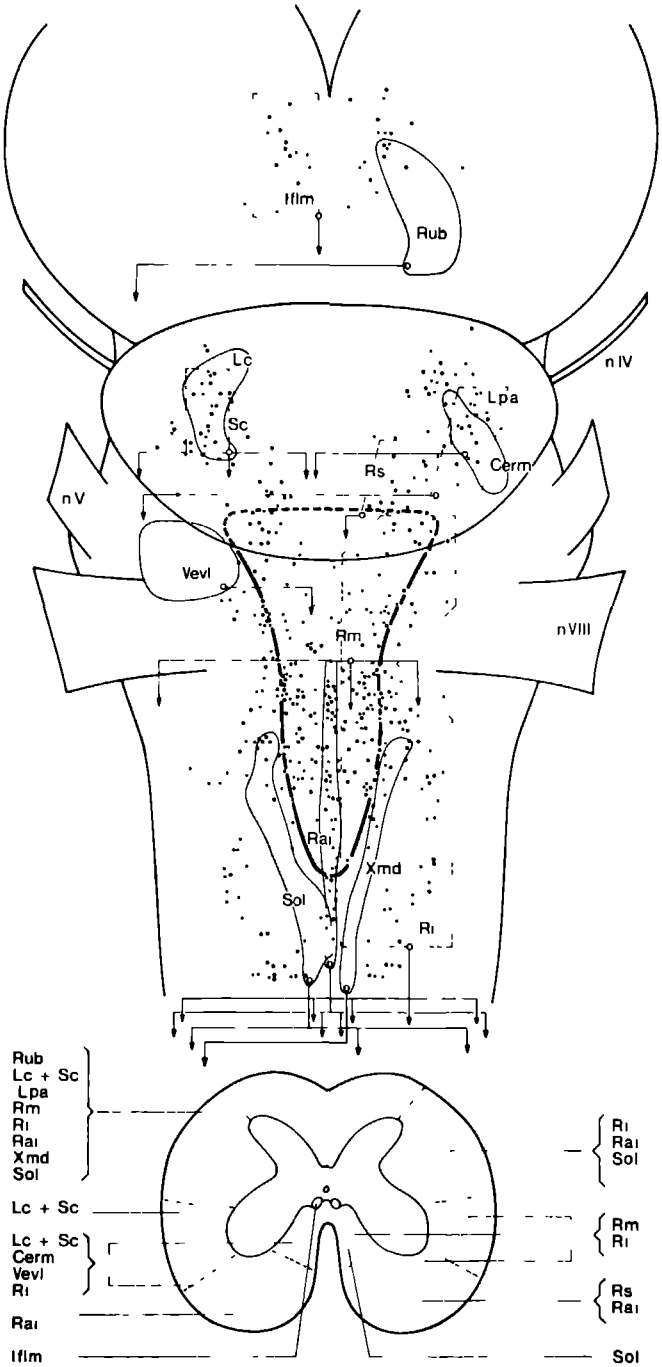


Fig 5. Dorsal view of the brain stem of the lizard *Varanus exanthematicus*, several well-defined nuclei (lines) and ill-defined cell masses (broken lines) have been projected in the horizontal plane Dots represent 1 out of 5 large (30–40 μm) and very large (> 40 μm) cell bodies in the magnocellular reticular formation Arrows near the midline indicate pathways descending through the ventral funiculus, whereas more lateral arrows represent pathways descending through the lateral funiculus Arrows crossing the midline represent contralateral projections In the transversal section of the spinal cord at level C7 the funicular trajectories of several descending pathways have been indicated

Maisky, 1977; Martin et al., 1979, 1981; Tohyama et al., 1979).

The nucleus raphes inferior fibers also show a wider funicular distribution than previously suggested. In addition to the spinal projection via the lateral funiculus, preferentially its dorsal part as already demonstrated in earlier studies (Ten Donkelaar, 1976a, b), a major raphe-spinal projection obviously passes via the ventral funiculus (Fig. 3F–H). Similar observations have been made in mammals (Kuypers and Maisky, 1977; Martin et al., 1978; Basbaum and Fields, 1979).

In the present study the funicular distribution of axons from the medial cerebellar nucleus, the ventrolateral vestibular nucleus, the dorsal motor nucleus of the vagus nerve and the nucleus of the solitary tract has been demonstrated. With respect to the medial cerebellar nucleus the present data suggest a predominantly contralateral spinal projection from this nucleus through the entire ventral funiculus (Figs. 3A and 4B), which is in keeping with results obtained in opossum (Martin et al., 1975) and monkey (Batton et al., 1977). In cat (Fukushima et al., 1977) and tree shrew (Ware and Mufson, 1979), however, a projection via the lateral funiculus was reported. The ventrolateral vestibular nucleus was found to project to the spinal cord via the ventral funiculus, in agreement with previous data (Robinson, 1969; Ten Donkelaar, 1976a,b; Cruce and Newman, 1981).

The spinal projection from the dorsal motor nucleus of the vagus nerve has been described in chicken (Smolen et al., 1977), opossum (Crutcher et al., 1978) and reptiles (Ten Donkelaar and de Boer-van Huizen, 1978; Ten Donkelaar et al., 1980; Cruce and Newman, 1981). The present study demonstrates its course via the dorsal part of the lateral funiculus (Fig. 1H). Spinal projections from the nucleus of the solitary tract pass via the dorsal part of the lateral funiculus (Fig. 1H), but also by way of the ventral funiculus (Fig. 4E–G).

It can be concluded that HRP slow-release gels provide a reliable technique to study the funicular trajectories of descending brain stem pathways. The results confirm much of the knowledge already gathered by other techniques, but suggest in some cases a wider funicular distribution than previously known.

SUMMARY

The funicular trajectories of various descending brain stem pathways in the lizard *Varanus exanthematicus* have been demonstrated, following a technique introduced by Griffin et al. (1979), using HRP slow-release gels. Following an implantation in the dorsal part of the lateral funiculus labeled cells were found in the contralateral red nucleus, the ipsilateral locus coeruleus and subcoeruleus area, a contralateral cell group presumably comparable to the mammalian lateral pontine area, bilaterally in the nucleus reticularis inferior and in the nucleus of the solitary tract, and in the nucleus raphes inferior. Hypothalamospinal fibers pass by way of a more ventral part of the lateral funiculus, as do some coeruleospinal fibers. After an HRP gel in the ventral funiculus labeled cells appeared in the subcoeruleus area, the contralateral medial cerebellar nucleus, the vestibular nuclear complex and throughout the magnocellular reticular formation, including the nucleus raphes inferior. Interstitiospinal fibers pass by way of a separate bundle in the dorsalmost part of the ventral funiculus.

In keeping with data in cat (Kuypers and Maisky, 1977; Tohyama et al., 1979) and opossum (Martin et al., 1979, 1981), our findings suggest a wider funicular distribution of various descending pathways than assumed in earlier studies.

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ABBREVIATIONS

Cerm	=	medial cerebellar nucleus	Rs	=	nucleus reticularis superior
flm	=	fasciculus longitudinalis medialis	Rub	=	nucleus ruber
Iflm	=	nucleus interstitialis of the flm	Sc	=	subcoeruleus area
Lc	=	locus coeruleus	Sol	=	nucleus of the solitary tract
nVI	=	nervus abducens	Vevl	=	nucleus vestibularis ventrolateralis
nVIII	=	nervus vestibulocochlearis	Vevm	=	nucleus vestibularis ventromedialis
Rai	=	nucleus raphes inferior	VI	=	nucleus n abducentis
Ras	=	nucleus raphes superior	Xmd	=	dorsal motor nucleus of the vagus
Ri	=	nucleus reticularis inferior	XII	=	nucleus n hypoglossi
Rm	=	nucleus reticularis medius			

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DISTRIBUTION OF CATECHOLAMINES IN THE BRAIN STEM AND SPINAL CORD OF THE LIZARD VARANUS EXANTHEMATICUS: AN IMMUNOHISTOCHEMICAL STUDY BASED ON THE USE OF ANTIBODIES TO TYROSINE HYDROXYLASE

Abstract—Antibodies to tyrosine hydroxylase were used to study the distribution of nerve cells, fibers and terminals, containing catecholamines, in the lizard *Varanus exanthematicus*, by means of the indirect immunofluorescence technique. Tyrosine hydroxylase-containing cell bodies occurred in the hypothalamus, the ventral and dorsal tegmentum mesencephali, the substantia nigra, the isthmic reticular formation, in and ventrolaterally to the locus coeruleus, in the nucleus tractus solitarii and in a lateral part of the nucleus reticularis inferior In addition tyrosine hydroxylase-containing cell bodies were found throughout the spinal cord, ventral to the central canal.

Tyrosine hydroxylase-immunoreactive terminal areas in the brain stem were seen in the nucleus interstitialis of the fasciculus longitudinalis medialis, the nucleus raphes superior, the locus coeruleus, several parts of the reticular formation and the nucleus descendens nervi trigemini

Ascending catecholaminergic pathways could be traced from the ventral mesencephalic tegmentum as well as from the dorsal isthmic tegmentum rostralwards, through the lateral hypothalamus. These pathways correspond to the mesostriatal and isthmocortical projections respectively, as described in mammals Furthermore, ascending catecholaminergic fibers could be traced from the catecholaminergic cell groups in the medulla oblongata to the isthmus, where they intermingle with the locus coeruleus neurons. These pathways correspond to the medullohypothalamic projection and to the dorsal periventricular system in mammals Descending catecholaminergic fibers to the spinal cord pass via the dorsomedial part of the lateral funiculus, and mainly terminate in the dorsal horn

The results obtained in the present study have been placed in a comparative perspective, which illustrates the constancy of catecholaminergic innervation throughout phylogeny.

Since Dahlström and Fuxe[33] in 1964 first described twelve groups of catecholamine (CA)-containing neurons (A1–A12) and nine groups of serotonin-containing neurons (B1–B9) in the rat brain, the distribution of monoaminergic nerve fibers and terminals as well as of monoamine-containing cell bodies has been studied in a wide variety of mammals (e.g. opossum,[31] rat,[10,45,57,66,99] cat,[14,29,72,83,115,155] rabbit,[13] tree shrew,[93] monkey,[41,50,61,65] man[16,96,98,112]) but also in lower vertebrates (bony fishes,[105,106,161] amphibians,[37,101,161] reptiles,[7,20,100,108,148,161] birds[36,49,144,161]).

Most of these studies have been carried out with the formaldehyde-induced fluorescence technique as developed by Falck and Hillarp[38–40] and its modifications.[43,75] This technique, however, has two major disadvantages. First, the fluorescence of serotonin-containing structures fades quite rapidly under the influence of ultraviolet light. Secondly, it is impossible to discern the different types of catecholamines such as dopamine, noradrenaline and adrenaline.

A different approach, the immunohistochemical visualization of transmitter synthesizing enzymes,[27,42,46,47,51,55] or transmitter substances themselves[131] are therefore more suitable for mapping central monoamine distribution, especially when distinction between the different monoamines is desired. This technique has recently found wide application for monoamine mapping purposes, particularly in mammals.[17–19,34,57,74,87,94,112,129,131,135,136,151–153] In lower vertebrates, however, immunohistochemical techniques have hardly been used with this aim.[35,60,117,132,134,148]

In the present study the indirect immunofluorescence technique is used to determine the localization of catecholamines, in the brain stem and spinal cord of a reptile, the lizard *Varanus exanthematicus*. An antiserum to tyrosine hydroxylase is used, since we may consider tyrosine hydroxylase

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Abbreviations: CA, catecholamine; CSF, cerebrospinal fluid; PBS, phosphate-buffered saline, TH, tyrosine hydroxylase

indicative for the presence of dopamine, noradrenaline and or adrenaline A comparable antiserum has also been used in a recent study in reptiles on striatotegmental projections [22a] An attempt was made to distinguish between dopamine and noradrenaline, using antibodies to dopamine β-hydroxylase as well (anti-bovine dopamine β-hydroxylase and anti-guinea pig dopamine β-hydroxylase) These experiments, however, remained unsuccessful Therefore, in the present study no distinction could be made between the various catecholamines This study forms part of a larger project concerning the organization of descending pathways from the brain stem to the spinal cord in this lizard [141 157] Horseradish peroxidase studies in various vertebrates, including reptiles,[5 25 32 53 70 88 95 126 140 141 147 157 160] have shown that several cell groups which project to the spinal cord, such as the locus coeruleus, the nucleus reticularis superior pars lateralis, the raphe nuclei, show a close resemblance to noradrenergic and serotonergic cell groups Characterizing these pathways as well as their cells of origin with respect to their neurotransmitters, is an important step in revealing the functional meaning of these descending systems The results of this study will be discussed in a comparative perspective

In a following paper the localization of serotonin in the brain stem and spinal cord of *Varanus exanthematicus* will be discussed (J G Wolters, H J ten Donkelaar, H W M Steinbusch and A A J Verhofstad, in preparation)

Preliminary reports of the present study were made to the European Neuroscience Association[159] and to the Anatomical Society of Great Britain and Ireland and the Nederlandse Anatomen Vereniging [158]

EXPERIMENTAL PROCEDURES

Experimental animals

Three lizards (*Varanus exanthematicus*) were used, varying in weight from 300 to 485 g, with a total length of 52–62 cm and a snout vent length of 26–31 cm The animals were housed in an air-conditioned room with a fixed temperature and dark–light cycle (10 h, 18 C/14 h, 24 C) One animal received colchicine (2 mg dissolved in 0 9% sodium chloride containing 2% vitamin C) 48 h before being sacrificed (40 μl in the cisterna magna and 200 μl intraperitoneally) An additional amount was injected intraperitoneally 24 h (400 μl) and 2 h (600 μl) before fixation

Preparation of tissues

The animals were intubated, anaesthetized with a mixture of oxygen, nitrous oxide and halothane, and perfused through the heart with ice-cold (4 C) calcium-free Tyrode's solution (Ca^{2+} replaced by Mg^{2+}) for 2 min, immediately followed by ice-cold (4 C) 4% paraformaldehyde dissolved in 0 1 M sodium phosphate buffer, pH 7 3,[113] for 20–30 min The brain and spinal cord were dissected out, postfixed for 2 h at 4 C in the same fixative and rinsed at 4 C in 5% sucrose in 0 1 M sodium phosphate buffer, pH 7 3, for 18–24 h The brain stem from the caudal level of the diencephalon up to the spinal cord and pieces taken at four levels of the spinal cord (viz the 6th, 15th, 26th and 38th spinal segments, representing respectively cervical, thoracic, lumbar and tail levels) were frozen with powdered carbon dioxide gas Transversal sections were cut at 10 μm on a cryostat (Dittes, Heidelberg G F R) Series of sections collected at 22 levels through the brain stem and the four levels of the spinal cord were mounted on glass slides coated with chrome alum gelatin (0 05 g chrome alum gelatin in 100 ml distilled water) and stored at −70 C pending staining In the brain stem the interval between the first sections of two consecutive series was about 500 μm

Immunofluorescence procedure

Two series of sections were stained according to the indirect immunofluorescence procedure described by Coons and collaborators[28] employing a rabbit antiserum to tyrosine hydroxylase (TH) isolated from rat adrenal glands or serum from non-immunized animals The isolation of TH and the characterization of the antiserum were carried out as described elsewhere [51 111] The sections were first rinsed in phosphate-buffered saline (PBS) at room temperature for 30 min Then they were incubated with the antiserum to TH or the non-immune serum both diluted 1 400 with PBS containing 0 1% Triton X-100[54] for 18 h at 4 C After rinsing in PBS for 30 min at room temperature the sections were incubated with fluorescein isothiocyanate-labeled sheep anti rabbit immunoglobulins (Statens Bakteriologiska Laboratorium Stockholm, Sweden) diluted 1 16 with PBS also containing 0 1% Triton X 100 (30 min room temperature) Finally, they were rinsed in PBS for 30 min at room temperature mounted in glycerin-PBS (3 1) and stored at −20 C

Evaluation and presentation of results

A Zeiss Universal microscope equipped for fluorescence with incident illumination was used for examination and photomicrography Photographs were taken with Kodak Tri-X film The distribution of immunoreactive cell bodies fibers and terminals is presented on schematic drawings of cryostat sections stained with cresylecht violet The density of fluorescent fibers and terminals was classified subjectively into five categories (a) no fluorescence (b) low density (c) medium density, (d) high density, (e) very high density

RESULTS

The distribution of CA-containing cell bodies, fibers and terminals will be described from rostral to caudal, starting at the level of the commissura posterior and ending in a tail segment of the spinal cord The terminology used is mainly based on Butler and Northcutt,[24] Cruce and Nieuwenhuys,[30] and ten Donkelaar and Nieuwenhuys [142]

Immunofluorescent cell bodies can easily be recognized (see e g Figs 12, 14, 19, 20 and 25), regardless of their individual morphology and dimensions, by the bright yellow-green fluorescein isothiocyanate fluorescence In the perikaryon often a dark spot is seen, which marks the place of the nucleus Immunoreactive cell bodies can be easily distinguished from lipofuscin autofluorescence, which has a more yellow-brownish colour and a more granular aspect However, it may sometimes be difficult to determine whether terminals or cross-sectioned immunoreactive fibers are seen,[44] especially when fine varicosities occur in a well confined restricted area

The results are not markedly different in colchicine-treated and untreated lizards, especially not for the distribution of immunoreactive fibers

However, in the spinal cord TH-immunoreactive neuronal cell bodies are only observed after colchicine pretreatment

Diencephalon

Only the caudal part of the diencephalon has been studied A small group of CA-containing cell bodies is located in the nucleus posterodorsalis, dorsolateral to the commissura posterior (Fig 1) In the dorsal part of the lateral hypothalamus, lateral to the nucleus periventricularis hypothalami, a major concentration of CA cells is found This cell cluster, intermingled between the fibers of the medial forebrain bundle, extends from the level of the posterior commissure to that of the rostral end of the nucleus opticus tegmenti (or nucleus of the basal optic root) (Figs 1, 12 and 13)

The axons and dendrites of the CA neurons in this cell group diverge mainly in lateral and ventrolateral directions and form the most impressive immunofluorescence at the mesodiencephalic junction (Figs 1 and 12) Ventrolaterally to these processes as well as interspersed in their medial part, longitudinally coursing CA-positive fibers are present, closely related to the lateral and the medial forebrain bundles, respectively (Fig 12) Thick cross-sectioned catecholaminergic fibers are visible in the dorsal part of the nucleus periventricularis hypothalami, whereas in its intermediate part a rather well-defined bundle of thin CA fibers can be observed (Fig 12) Furthermore terminal-like varicosities are present in this nucleus The intense immunofluorescence in the median eminence (Figs 1 and 12) probably represents the dense CA innervation by the tuberohypophyseal system The cells of origin of this projection may be found among the neurons of the CA cell group in the dorsolateral hypothalamus

Mesencephalon

Some scattered small to medium-sized immunofluorescent catecholaminergic cells are seen in the nucleus laminaris of the torus semicircularis, at the level of the nucleus of Edinger–Westphal (Fig 2) These cells are not continuous with the CA cell group in the lateral hypothalamus In the area ventral and medial to the red nucleus, a considerable number of CA-positive cells is found between the fibers of the oculomotor nerve (Fig 2) This cell area has been termed the ventral tegmental area, based on its relative position and fiber connections [139] More caudally this cell group is found around the interpeduncular nuclei (Figs 3, 14, 16 and 18), and fades at the level of the caudal end of the nucleus oculomotorius (Figs 4 and 17) This cell group is continuous with the numerous round or oval medium-sized catecholaminergic cells in the substantia nigra (Figs 3, 14 and 15) Just caudally to the latter a group of CA-positive cell bodies is found, extending from the dorsocaudal pole of the substantia nigra, through the isthmic reticular formation, into the central grey along the aqueductus cerebri (Figs 3, 4, 17 and 19) In the mesencephalon also some scattered CA-containing cells are present in the nucleus interpeduncularis dorsalis

Between the fibers of the oculomotor nerve, the immunofluorescent processes of the CA-positive neurons in the ventral tegmental area are visible as well as cross-sectioned varicose fibers, the latter chiefly in the ventrolateral part of this area (Fig 2) These cross-sectioned fibers most probably originate from the catecholaminergic cell groups of the substantia nigra and the ventral tegmental area, which send their processes mainly in the ventromedial direction, forming the beginning of the nigrostriatal pathway and the mesolimbic projection, respectively Transversely cut CA nerve fibers occur throughout the interstitial nucleus of the fasciculus longitudinalis medialis and the nuclei of the torus semicircularis, with a condensation ventral to the laminar nucleus, just lateral to the oculomotor nucleus and the nucleus of Edinger–Westphal (Fig 2) This condensation may represent the dorsal noradrenergic bundle arising from the locus coeruleus and projecting to the telencephalon, i e the isthmocortical path [104] In the tectum mesencephali the stratum griseum centrale receives a sparse catecholaminergic innervation (Figs 1 4) In the caudal midbrain (Figs 3 and 4) the most conspicuous TH-immunofluorescent fibers are the processes of the TH-positive neurons in the substantia nigra (Figs 14 and 15), and those of the more caudally located cells in and ventral to the central grey (Figs 17 and 19) Dorsally and medially to these processes, respectively, cross-cut fibers are seen, probably those of the dorsal noradrenergic bundle In the nucleus interpeduncularis dorsalis, especially in its dorsal part, cross-sectioned CA fibers are also present (Figs 3, 4, 14 and 16–18) In parts of the nucleus centralis of the torus semicircularis (Figs 3 and 4), which is the reptilian homologue of the mammalian colliculus inferior, weakly fluorescent fine varicosities and CA-containing fibers are seen

Isthmic level

Medium-sized TH-immunoreactive cell bodies are found in the locus coeruleus, and in the area ventrolateral to this nucleus (Figs 5 and 20–22) The richly branching processes of the (most likely noradrenergic) locus coeruleus neurons form an impressive fluorescent plexus, which sends its varicose as well as non-varicose fibers in all directions, preferentially to the lateral edge of the brain stem (Figs 20 and 21) The same holds for the processes of the CA neurons in the lateral subcoeruleus area (Figs 20 and 22) Fine brightly fluorescent varicosities are seen between the CA fibers in and surrounding the locus coeruleus (Fig 21) Weakly fluorescent, very fine varicosities occur ventromedially to the locus coeruleus and in the area ventral to the fasciculus

longitudinalis medialis bordering the lateral confines of the nucleus raphes superior (Fig 20)

Medulla oblongata

Tyrosine hydroxylase-immunoreactive perikarya are only found at the level of the obex One cell group lies in the dorsal part of the nucleus of the solitary tract, another in a lateral part of the reticular formation (Figs 7 and 23) The processes of the former neurons are oriented in the horizontal plane, those of the latter cells are more or less vertically arranged (Figs 23 and 25) In the rostral part of the medulla oblongata weakly fluorescent very fine varicosities occur ventrolaterally to the nucleus of the abducens nerve, and scattered through the fasciculus longitudinalis medialis, probably representing cross-cut descending noradrenergic connections to the caudal medulla oblongata and the spinal cord (Fig 6) Varicosities of the same type also occur in parts of the superior olive, the nucleus reticularis medius and in the dorsal part of the nucleus descendens nervi trigemini (Fig 6) In the caudal medulla fine varicosites surround the canalis centralis, and are found lateral to the dorsal motor nucleus of the vagus (Figs 7, 23 and 24), in the ventral part of the nucleus descendens nervi trigemini, as well as in the ventrolateral part of the fasciculus longitudinalis medialis (Figs 7 and 23), the latter may represent descending CA pathways to the spinal cord

Spinal cord

Bipolar catecholamine-containing cell bodies are found throughout the spinal cord located just ventral to the central canal (Figs 8 11 and 26 28), contacting the cerebrospinal fluid (CSF) by means of a fairly thick, straight process of variable length that reaches the ependymal layer A thin and often branching short process is directed ventralwards where it disappears between the interstitiospinal fibers situated in the dorsal part of the ventral funiculus The number of these CA neurons is limited in the transversal sections studied (thickness 10 μm) only one or two cells were present

Catecholaminergic nerve terminals occur in the dorsal horn of the grey matter throughout the spinal cord They are not clearly restricted to particular areas as distinguished by Kusuma *et al* [69] although they show a preference for the areas I and II, the medial part of the dorsal horn and the dorsal part of area X (Figs 27 and 28) Cross-sectioned descending TH-immunoreactive fibers are present in the dorsomedial part of the lateral funiculus at thoracic and lumbar levels, but are most distinct in the tail segments (Figs 8–11 and 26) In the ventral horn practically no TH immunoreactivity was observed

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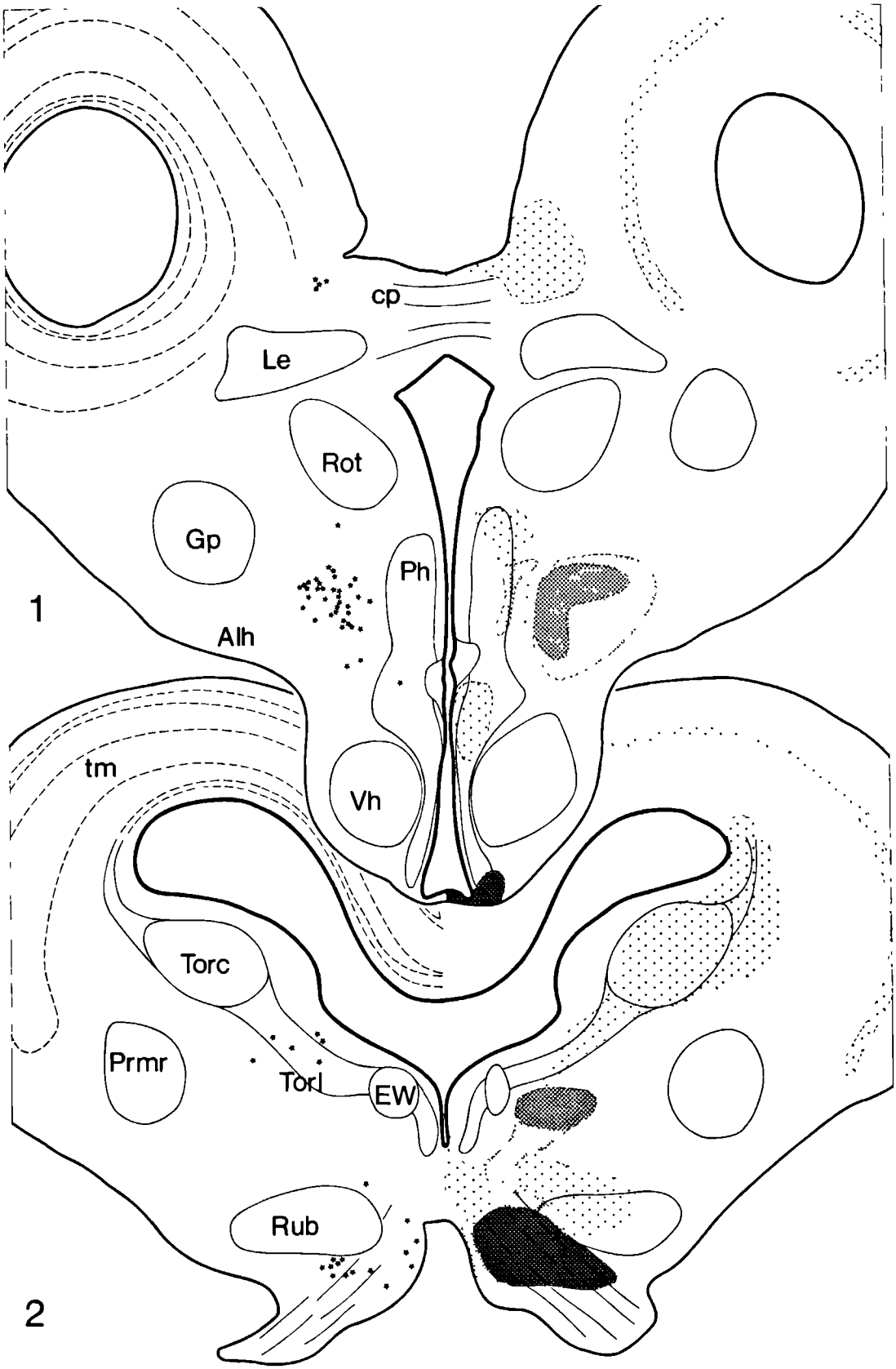
Figs 1–11 Schematic representation of TH immunoreactivity as observed in representative transversal sections of the brain stem and spinal cord of colchicine-treated lizards At the right, the density of immunoreactive fibers and varicosities is indicated, subjectively classified into five categories no fluorescence low density medium density, high density and very high density At the left and in the midline asterisks indicate immunofluorescent cell bodies in each section

Abbreviations used in figures

Alh	area lateralis hypothalami
cb	cerebellum
cc	canalis centralis
cd	cornu dorsale
Cerl	nucleus cerebellaris lateralis
Cerm	nucleus cerebellaris medialis
cp	commissura posterior
cv	cornu ventrale
Em	eminentia mediana
EW	nucleus of Edinger–Westphal
fl	funiculus lateralis
flm	fasciculus longitudinalis medialis
Fun	nucleus funiculi dorsalis
Gc	griseum centrale
gl	lamina granularis cerebelli
Gp	nucleus geniculatus pretectalis
Ico	nucleus intercollicularis
Ipd	nucleus interpeduncularis pars dorsalis
Ipv	nucleus interpeduncularis pars ventralis
Lc	locus coeruleus
Le	nucleus lentiformis pars extensa
Ll	nucleus lemnisci lateralis
ml	lamina molecularis cerebelli
Ols	nucleus olivaris superior
Ph	nucleus periventricularis hypothalami
Prmc	nucleus profundus mesencephali pars caudalis
Prmr	nucleus profundus mesencephali, pars rostralis
Pvo	paraventricular organ
Rai	nucleus raphes inferior
Ri	nucleus reticularis inferior
Rm	nucleus reticularis medius
Rot	nucleus rotundus
Rs	nucleus reticularis superior
Rub	nucleus ruber
Sc	nucleus subcoeruleus
Sn	substantia nigra
Sol	nucleus tractus solitarii
tm	tectum mesencephali
Torc	torus semicircularis, necleus centralis
Torl	torus semicircularis, necleus laminaris
Vds	nucleus descendens nervi trigemini
Vetg	nucleus vestibularis tangentialis
Vevl	nucleus vestibularis ventrolateralis
Vh	nucleus ventralis hypothalami
Vta	area tegmentalis ventralis
IIId	nucleus nervi oculomotorii, pars dorsalis
IIIi	nucleus nervi oculomotorii pars intermedia
VI	nucleus nervi abducentis
XII	nucleus nervi hypoglossi
Xmd	nucleus motorius dorsalis nervi vagi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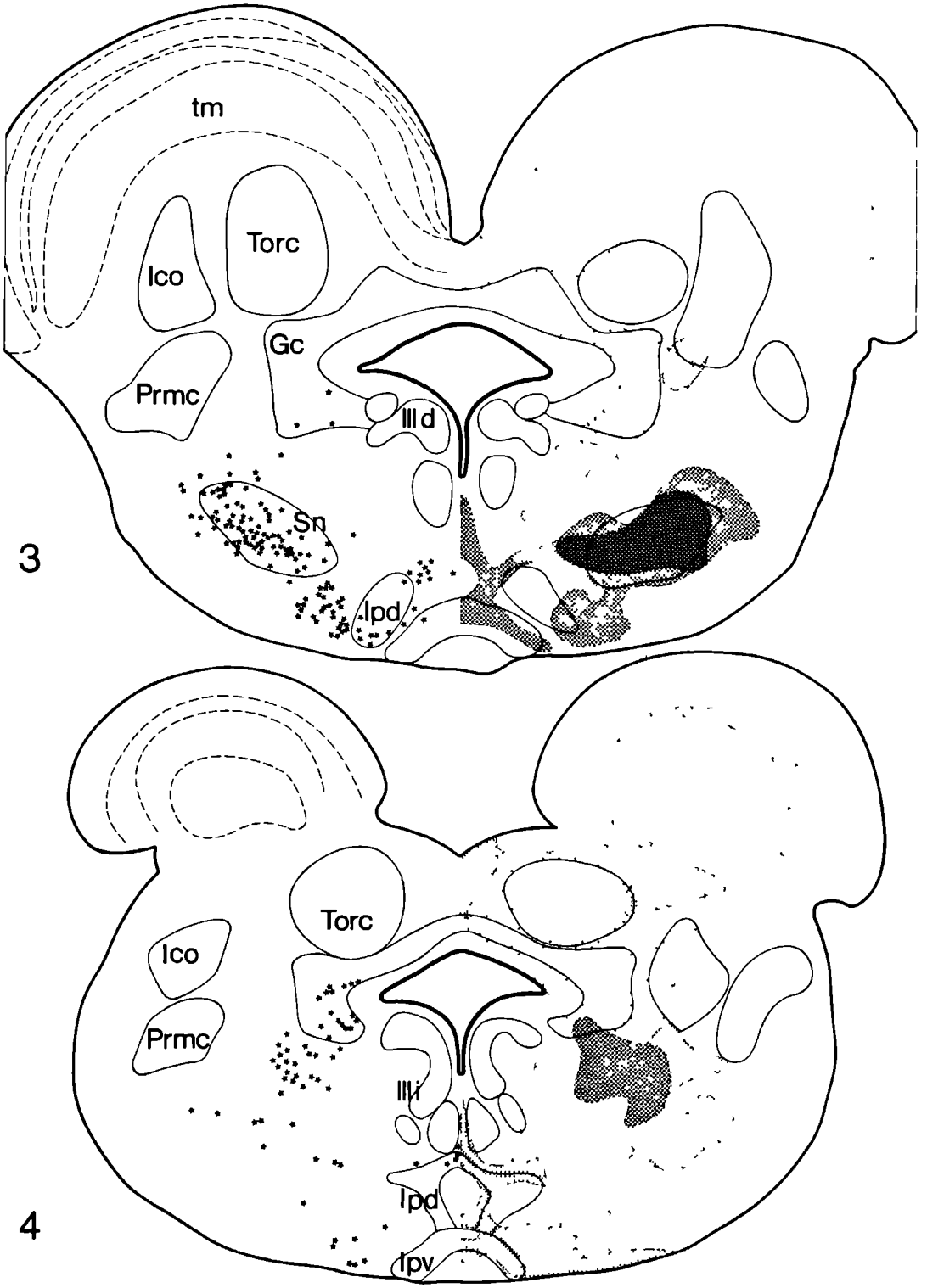
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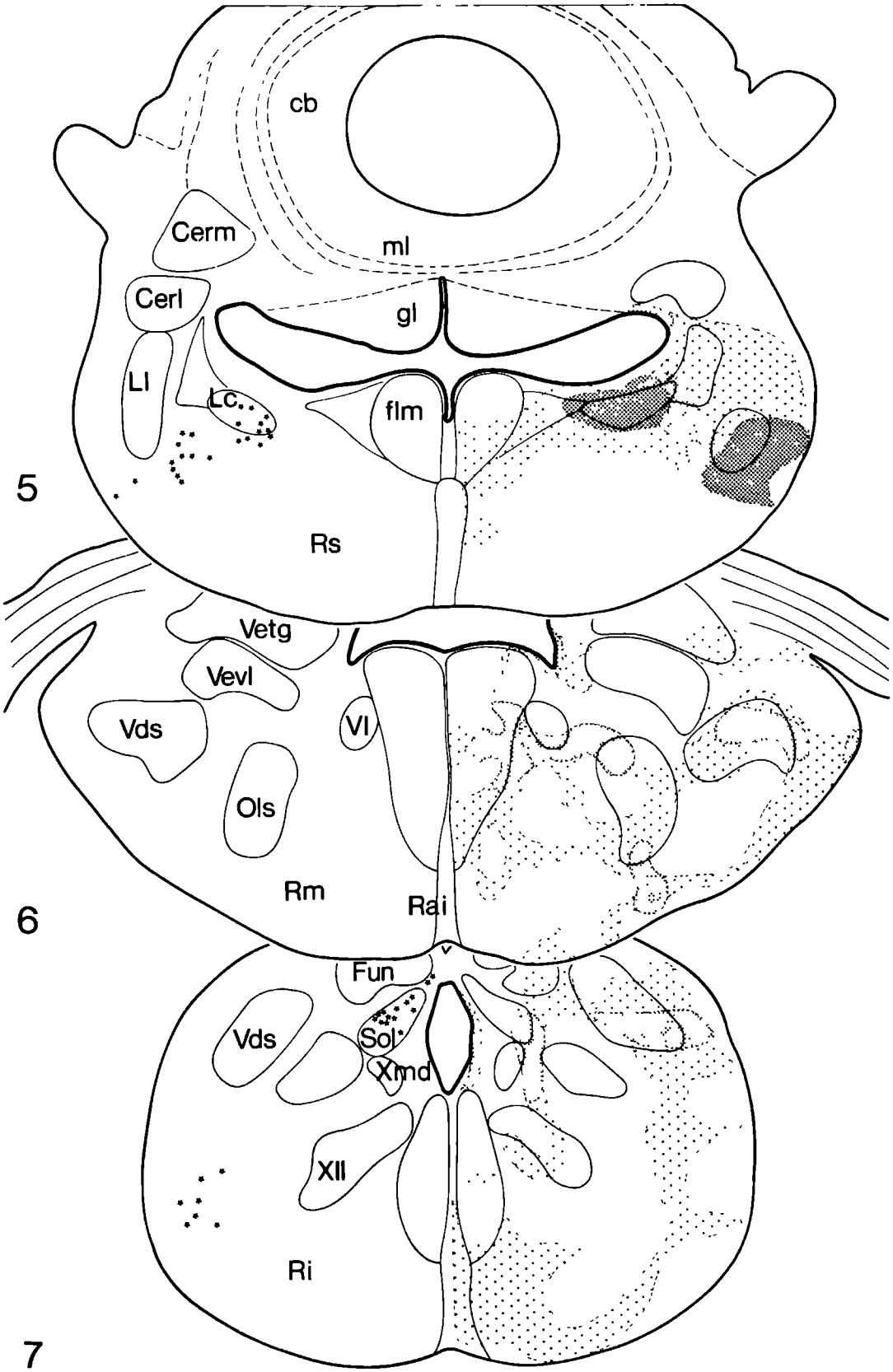
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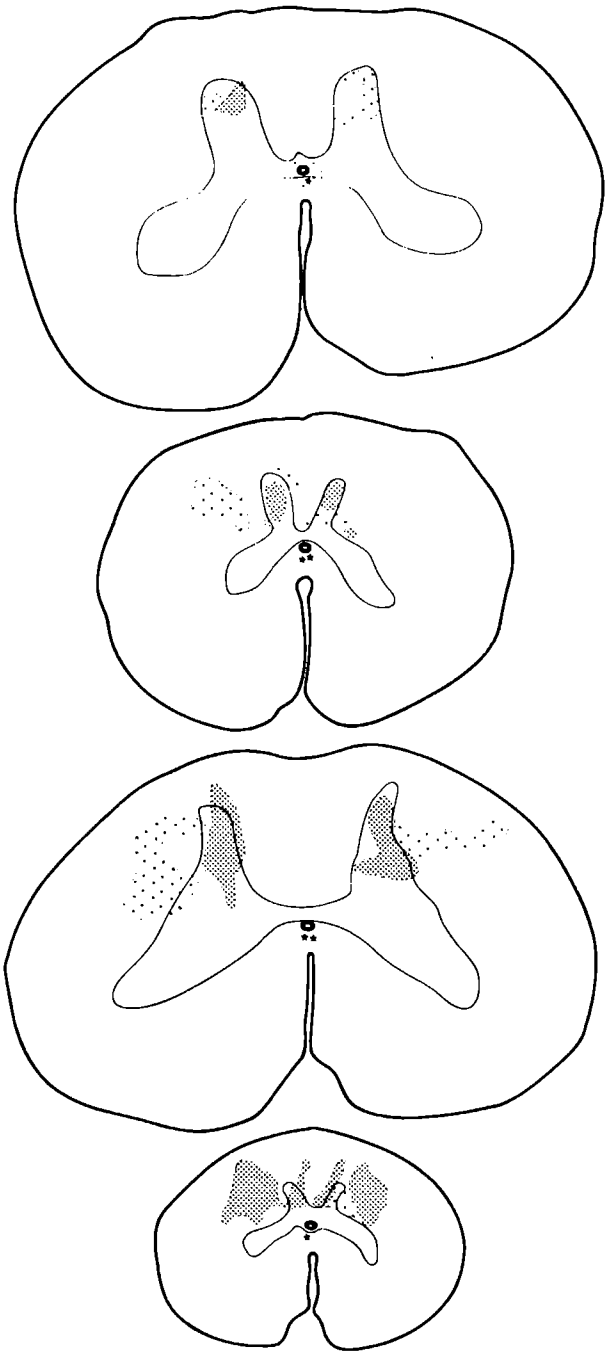
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DISCUSSION

The use of the indirect immunofluorescence technique has provided a much more complete picture of the catecholaminergic innervation of the brain stem and spinal cord in reptiles than was known before [7,20,22,100,104,108,161]. The present study shows that most of the CA-containing cell groups as originally described by Dahlström and Fuxe[33] in the rat brain stem, are also found in the brain stem of the lizard *Varanus exanthematicus*. Furthermore, various CA fiber tracts and terminal fields that occur in the mammalian brain[44,45,76,77,91,92,135,149] have also been observed in this lizard.

Distribution of catecholamine neurons in the brain stem and spinal cord

Prior to a discussion of our results, a brief outline of the CA cell groups in the mammalian brain stem and diencephalon will be presented.

The CA neurons in mammals are commonly divided into the following cell systems.[77,91,92] (1) a lateral tegmental cell system, composed of a medullary part (groups A1 and A3 of Dahlstrom and Fuxe[33]) which is located in the ventrolateral part of the medullary tegmentum, and a pontine part (A5 and A7), distributed from the level of the rostral part of the facial nucleus up to the parabrachial nuclei; (2) a dorsal medullary cell system (A2), found in the nucleus of the solitary tract and the dorsal motor nucleus of the vagus; (3) the locus coeruleus cell group, which includes the densely packed cells of the locus coeruleus proper (A6) and some cells laterally and dorsally along the medial aspects of the superior cerebellar peduncle into the roof of the fourth ventricle, i.e. group A4, (4) an extensive mesencephalic dopamine cell system, which can be divided into three parts: cells located in the substantia nigra (A9), cells located in the ventromedial mesencephalic tegmentum (A10), particularly in the ventral tegmental area of Tsai; cells extending caudally and dorsally from the substantia nigra into the ventrolateral midbrain tegmentum (A8), (5) a midline and periventricular cell system, associated with the periventricular CA fiber systems, cell bodies are found along the mesencephalic periaqueductal grey and periventricular grey of the caudal thalamus (A11), and in part also in group A10, (6) various diencephalic cell systems (see also Ref. 9) i.e the dopaminergic incertohypothalamic system (mainly groups A13 in the zona incerta, just dorsal to the dorsomedial hypothalamic nucleus, and A14, a rostral periventricular group in the anterior hypothalamus and preoptic region), and the dopamine cells of the tuberohypophyseal system (A12), situated in the hypothalamic arcuate nucleus.

In the brain stem and diencephalon (only its caudal part was studied) of the lizard *Varanus exanthematicus* various comparable cell systems were observed. The cluster of CA cells in the ventrolateral part of the caudal brain stem (see Figs 7 and 23) is comparable to the medullary component of the lateral tegmental system. The pontine part of this system is represented in the lizard *Varanus exanthematicus* by the cell group ventrolateral to the locus coeruleus. This cell group is comparable to A7. No caudal extension of this cell area to the level of the superior olive (see Fig. 6) was found, as group A5 in mammals. However, Parent and Poirier[108] noted in the turtle *Chrysemys picta* catecholamine-containing perikarya, scattered or in small groups in the lateral part of the reticular formation, also at the level of the superior olive. Brauth and Kitt[22] found CA-positive cell bodies at the level of the trigeminal motor nucleus in *Caiman crocodilus*. Horseradish peroxidase studies in reptiles[95,138,141] showed spinal projections of neurons situated in the ventrolateral part of the rostral rhombencephalic tegmentum, which might be comparable to spinal projections of A7 and A5 neurons in mammals.[11,19,81] Possibly the CA cells of the isthmic tegmentum in *Varanus exanthematicus* represent both A7 and A5 groups.

The cell group observed in or close to the nucleus of the solitary tract (Figs 7, 23 and 25) which has also been described in turtles,[104,108,161] is directly comparable to the A2 cell group observed in mammals.

The most distinct CA cell group in the rhombencephalon of the lizard *Varanus exanthematicus* is formed by the locus coeruleus (Figs 5, 20 and 21) as also observed in turtles,[104,108,161] and *Caiman crocodilus*;[22] this cell group is comparable to group A6 in mammals. Its ascending and descending projections will be discussed later. No lateral or dorsal extension of the locus coeruleus, comparable to group A4, is present in reptiles.

The most impressive CA cell system in the lizard *Varanus exanthematicus* is found in the mesencephalon (Figs 2–4, 14–17 and 19). Three, presumably dopaminergic[104] cell groups could be distinguished, i.e. the substantia nigra, a cell group medial to it and extending rostralward, comparable to the ventral tegmental area as observed by ten Donkelaar and de Boer-van Huizen,[139] and a caudodorsal extension of the substantia nigra. These cell clusters are comparable to groups A9, A10 and A8 of mammals, respectively. In the turtles *Chrysemys picta*[104,108] and *Chrysemys scripta*[22a] also a cluster of catecholaminergic cells has been found in the mesencephalic tegmentum which can be divided in a medial and a lateral part, comparable to the ventral tegmental area and substantia nigra of the present study, respectively. In *Caiman crocodilus*[22,22a] a large cluster of CA-positive cells was observed in the nucleus tegmenti pedunculopontinus as well as some cells medial to it, which might be comparable to the ventral tegmental area as observed in *Varanus exanthematicus*. The caudodorsal extension of the substantia nigra, comparable to A8, was not observed in turtles or crocodilians.

In the rostral part of the mesencephalon (see Fig. 2) some CA-positive cells are found in the periventricular grey and the laminar nucleus of the torus semicircularis, as well as a few in the periventricular hypothalamic grey. These cells may be comparable to the midline and periventricular cell system (A11) as found in mammals. Catecholamine-containing neurons in the nucleus posterodorsalis, dorsal to the posterior commissure, as found in *Varanus exanthematicus* (Fig. 1), have not been observed in other vertebrates.

Fig 12 Hypothalamus Tyrosine hydroxylase-immunoreactive neurons and their processes in the dorsal part of the lateral hypothalamus (group A13) in a non colchicine treated lizard Bundles of cross sectioned immunofluorescent fibers can be observed ventrolaterally to this cell cluster and in its medial portion as well as in several parts of the nucleus periventricularis hypothalami The very dense CA innervation of the median eminence is clearly visible Note that no TH immunoreactive neurons are present in the paraventricular organ (Bar 150 μm)

Fig 13 Hypothalamus, detail of Fig 12 Tyrosine hydroxylase-immunofluorescent neurons in the dorsolateral hypothalamus (group A13) In many of these cell bodies a dark spot indicates the location of the nucleus Note, that the fibers seen in this area exhibit a wide variety in thickness and that there are varicose as well as non varicose fibers (Bar 60 μm)

Fig 14 Caudal mesencephalon Tyrosine hydroxylase-immunoreactive cell bodies of the substantia nigra (group A9) and the ventral tegmental area (group A10) in a non colchicine treated lizard A broad bundle of cross-sectioned thin fluorescent fibers is visible dorsomedial to the substantia nigra Transversely cut thicker fibers can be seen ventrolateral and ventromedial to the CA cell group of the ventral tegmental area (Bar 150 μm)

Fig 15 Caudal mesencephalon, detail of Fig 14 Medium-sized TH-containing probably dopaminergic neurons of the substantia nigra (group A9) (Bar 60 μm)

Fig 16 Caudal mesencephalon, detail of Fig 14 Small and medium-sized TH-containing probably dopaminergic neurons of the ventral tegmental area (group A10) and several cross-cut fluorescent fibers in between and lateral to these cells (Bar 60 μm)

Fig 17 Caudal mesencephalon, just caudal to the plane of Fig 14 but rostral to the isthmomesencephalic junction A distinct TH-immunoreactive cell cluster is seen in the central grey near the aqueductus cerebri and some scattered TH-containing neurons can be observed in the nucleus interpeduncularis dorsalis of a non colchicine-treated lizard A multitude of fluorescent omnidirectional neuronal processes as well as cross-cut fibers is present mainly in the dorsal part of the isthmic reticular formation Thicker cross-sectioned fibers are present in the nucleus interpeduncularis dorsalis (Bar 150 μm)

Fig 18 Caudal mesencephalon detail of Fig 17 Numerous cross-sectioned TH-containing fibers and a few immunoreactive cell bodies present in the nucleus interpeduncularis dorsalis (Bar 60 μm)

Fig 19 Caudal mesencephalon, detail of Fig 17 The TH-immunoreactive cell cluster in and ventral to the central grey (group A8), which is continuous with the dorsolateral pole of the substantia nigra its neuronal processes, and passing fibers Note the variability of the fiber thickness Also fine and very fine varicosities can be discerned (Bar 60 μm)

Fig 20 Isthmic tegmentum, dorsal part The TH-immunoreactive, most probably noradrenergic neurons of the locus coeruleus (group A6) and subcoeruleus (group A7), in a non colchicine-treated lizard and the impressive fluorescent fiber plexus formed by the multidirectional neuronal processes of the locus coeruleus cell group, and the mainly horizontally oriented fibers lateral to it (Bar 150 μm)

Fig 21 Isthmic tegmentum, detail of Fig 20 Some TH-immunoreactive neurons of the locus coeruleus, surrounded by a plexus of omnidirectional immunofluorescent fibers (Bar 45 μm)

Fig 22 Isthmic tegmentum, detail of Fig 20 Two TH-immunoreactive cells of the nucleus subcoeuleus, surrounded by mainly horizontally coursing fibers Sparsely very thin varicose fibers are seen (Bar 45 μm)

Fig 23 Caudal medulla oblongata Tyrosine hydroxylase-immunoreactive neurons of the nucleus tractus solitarii (group A2) and of the lateral tegmental cell system (group A1), in a non colchicine-treated lizard (Bar 150 μm)

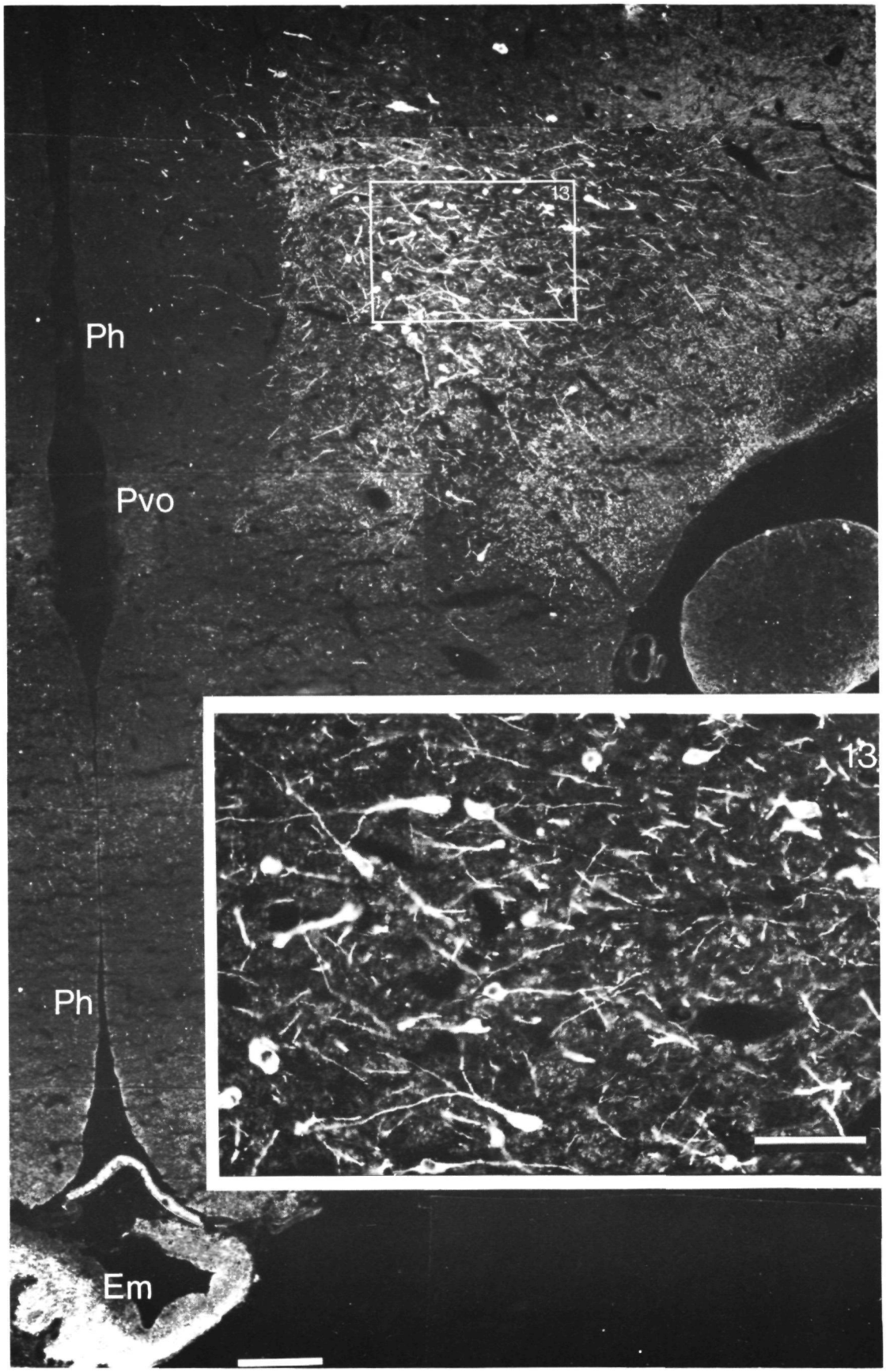
Fig 24 Caudal medulla oblongata, detail of Fig 23 A circumscribed area of fine varicosities ventral to the CA cells of the nucleus of the solitary tract, most probably representing the caudal part of the central tegmental tract, i e medullohypothalamic projections originating in the catecholaminergic cell groups A1 and A2 (Bar 75 μm)

Fig 25 Caudal medulla oblongata, detail of Fig 23 Tyrosine hydroxylase-immunoreactive neurons of the nucleus of the solitary tract (group A2) Note the horizontal orientation of their processes (Bar 60 μm)

Fig 26 Cervical intumescence of the spinal cord Two bipolar TH-immunoreactive neurons, located just ventral to the central canal, in a colchicine-treated lizard Several very thin fibers and varicosities are seen in the dorsal part of area X (Bar 45 μm)

Fig 27 Lumbar intumescence of the spinal cord Two bipolar TH-immunoreactive neurons, located just ventral to the central canal in a colchicine-treated lizard Note the long apical process, contacting the ependymal surface of the central canal In mainly the dorsal and central parts of area X varicosities and a few very thin fibers can be observed (Bar 45 μm)

Fig 28 Tail segment of the spinal cord Cross-sectioned TH-immunoreactive fibers and varicosities are seen in the dorsomedial part of the lateral funiculus and in the dorsal horn Ventral to the central canal a CA neuron is seen Picture is taken from a colchicine-treated lizard (Bar 60 μm)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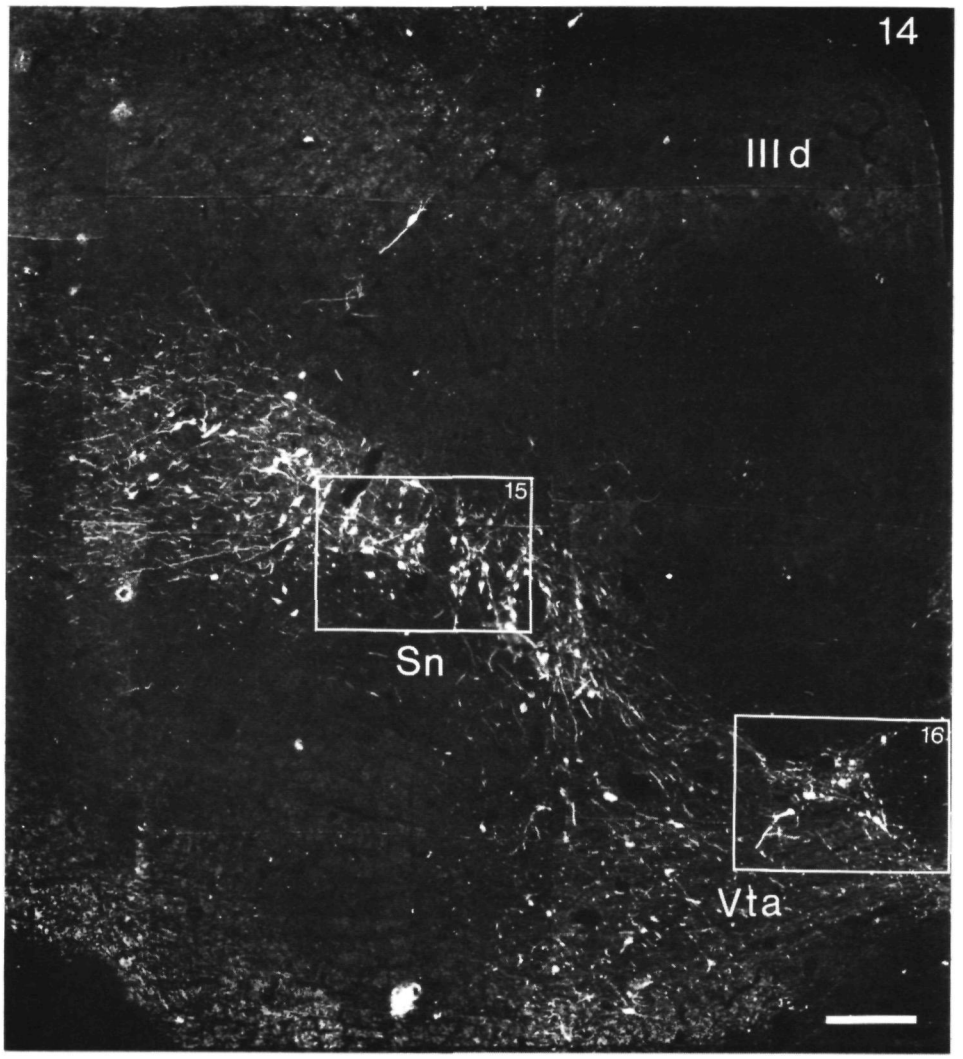
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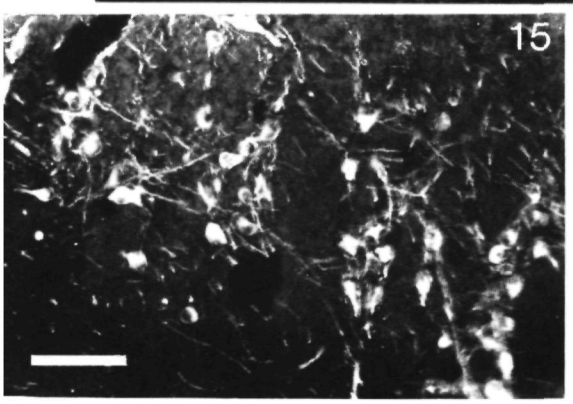
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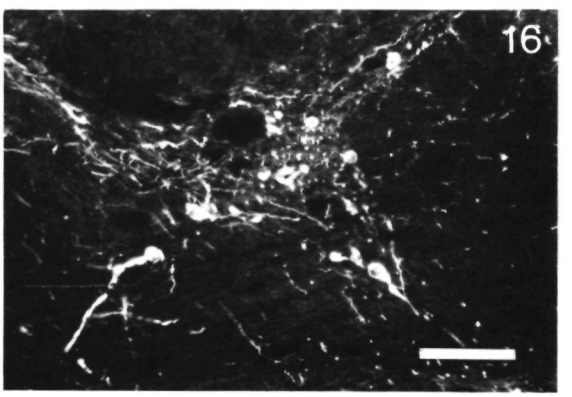
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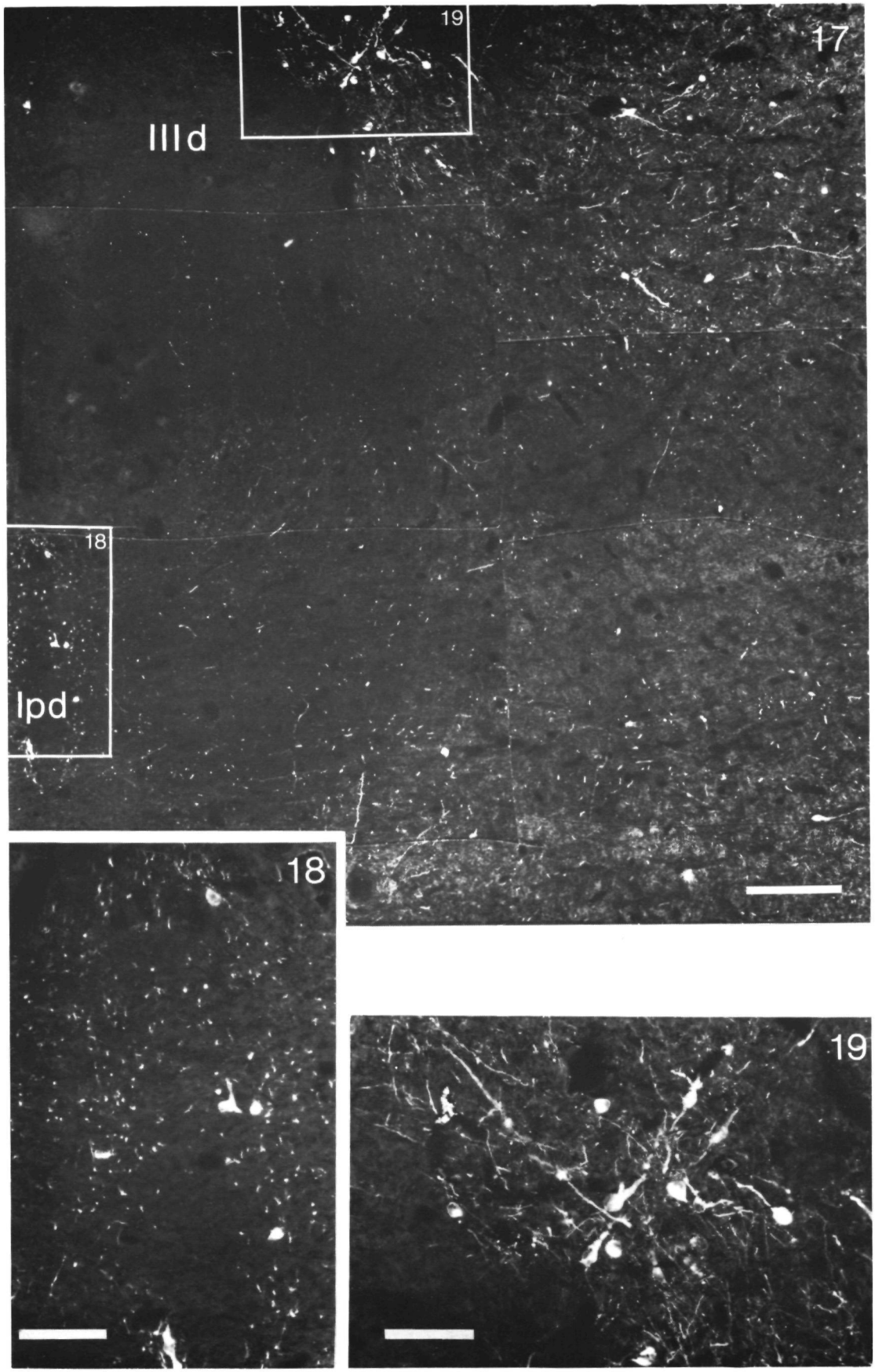
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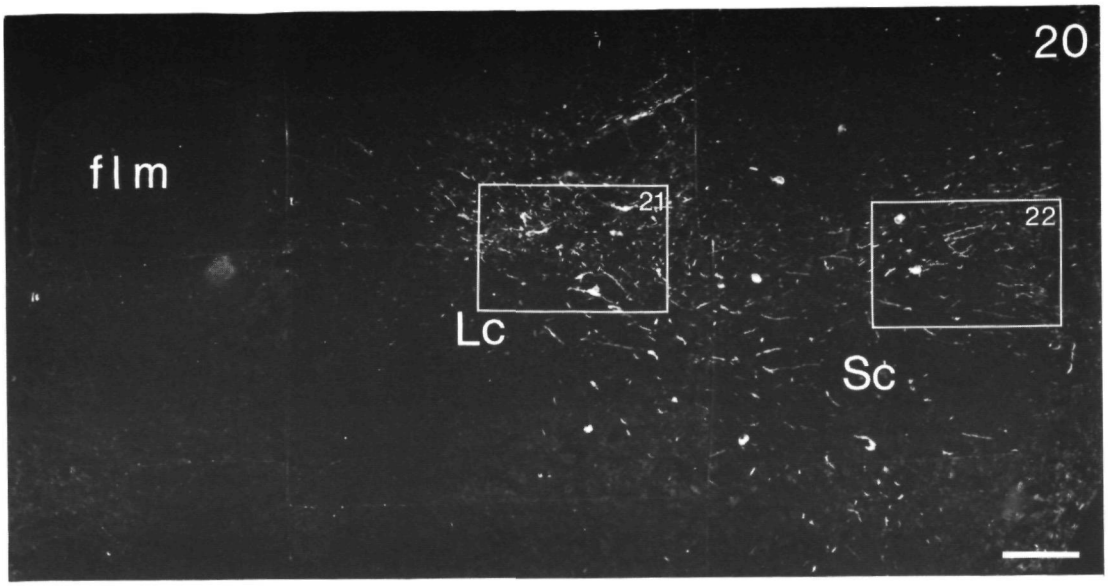
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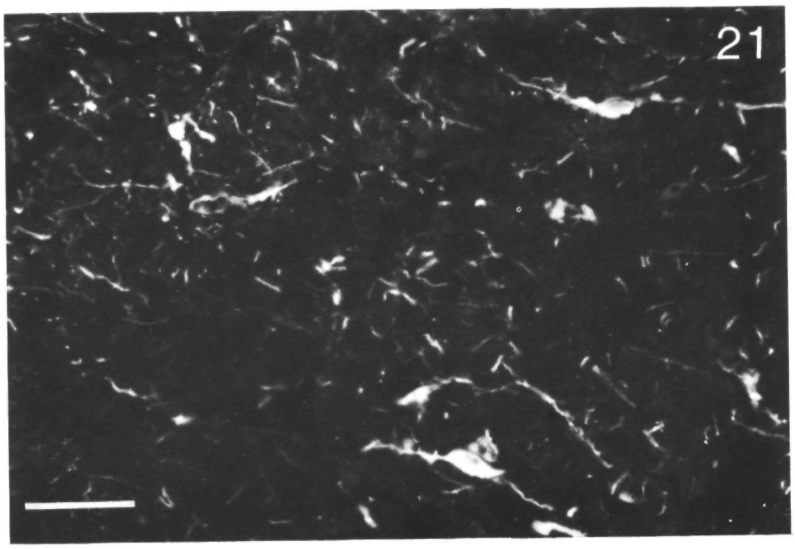
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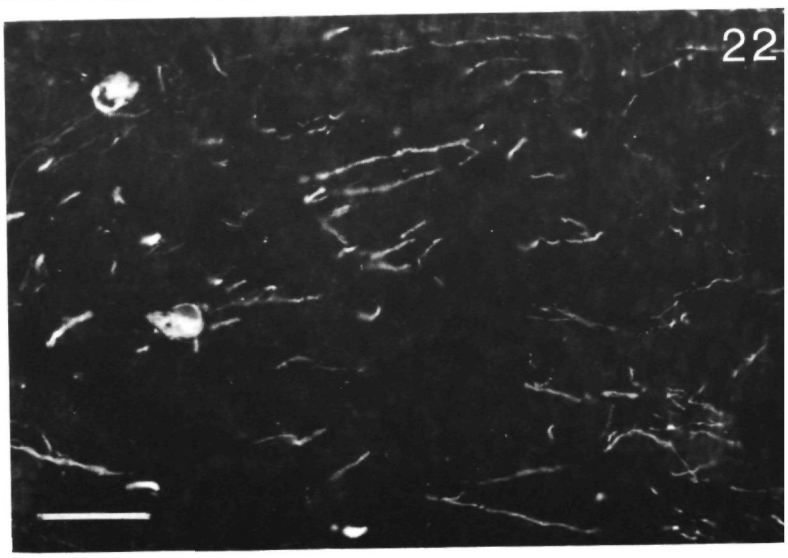
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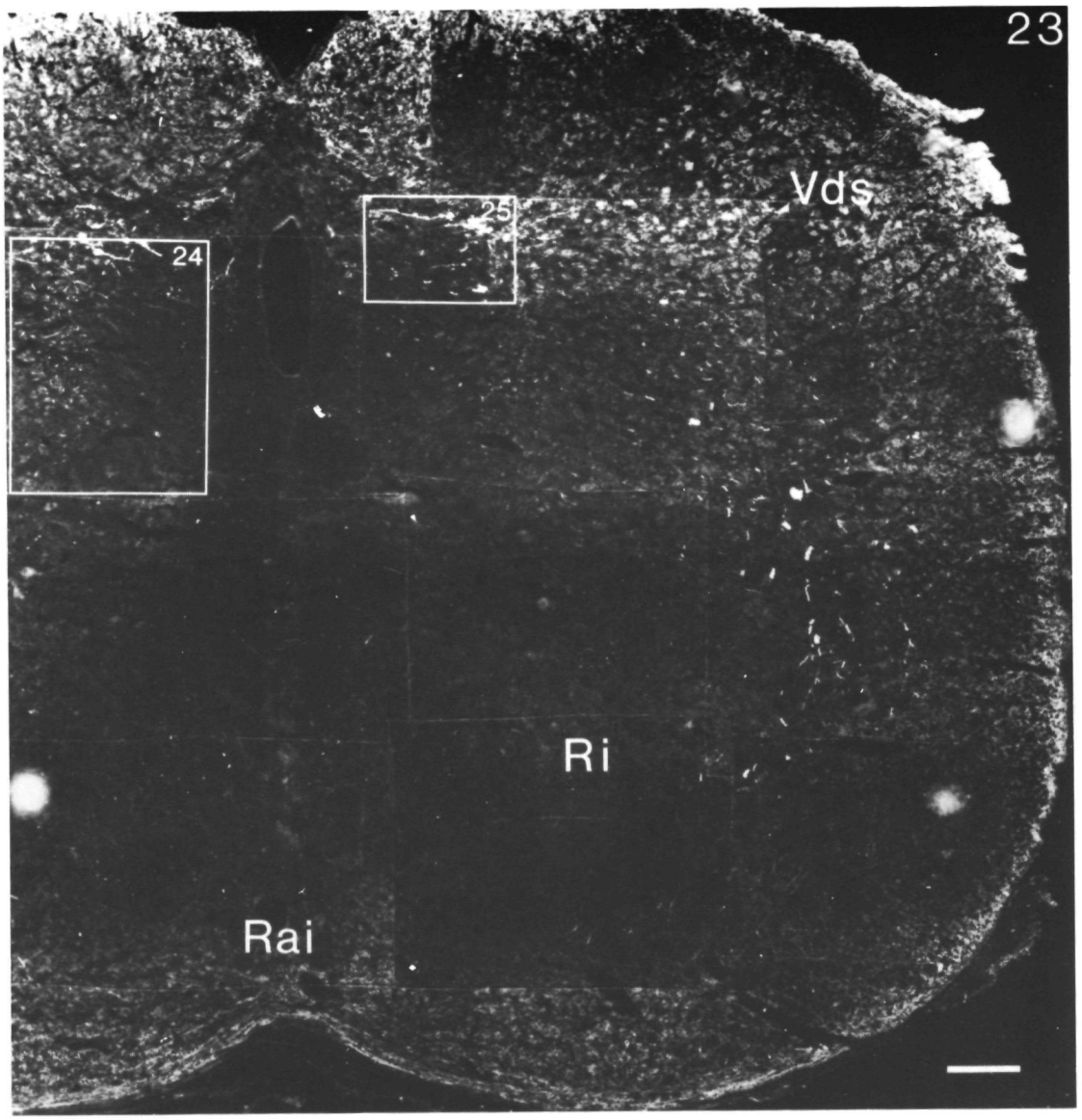
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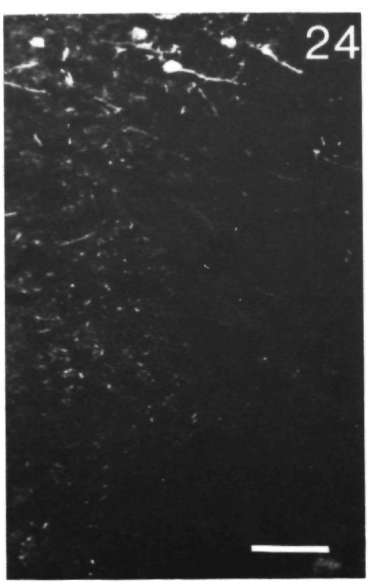
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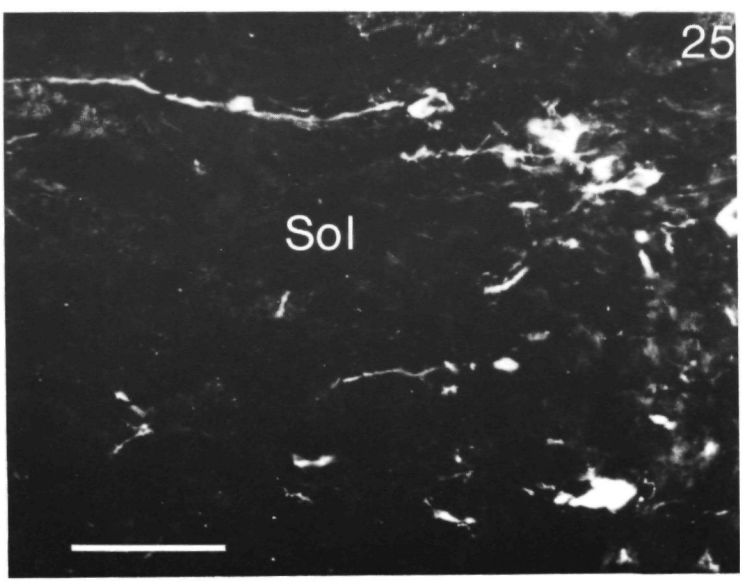
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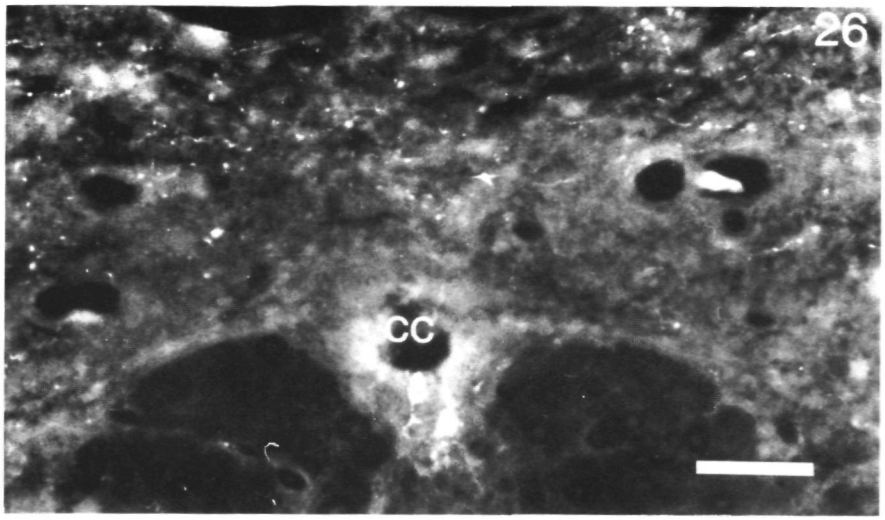
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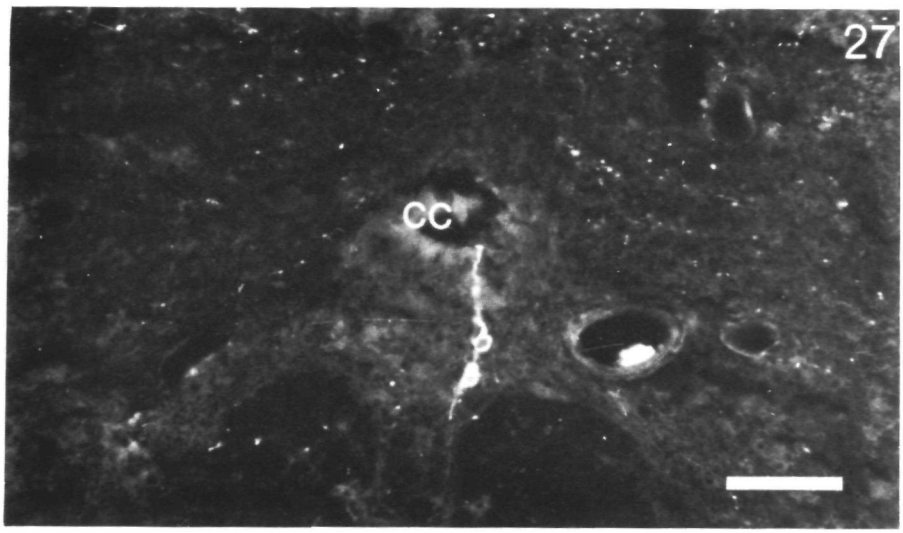
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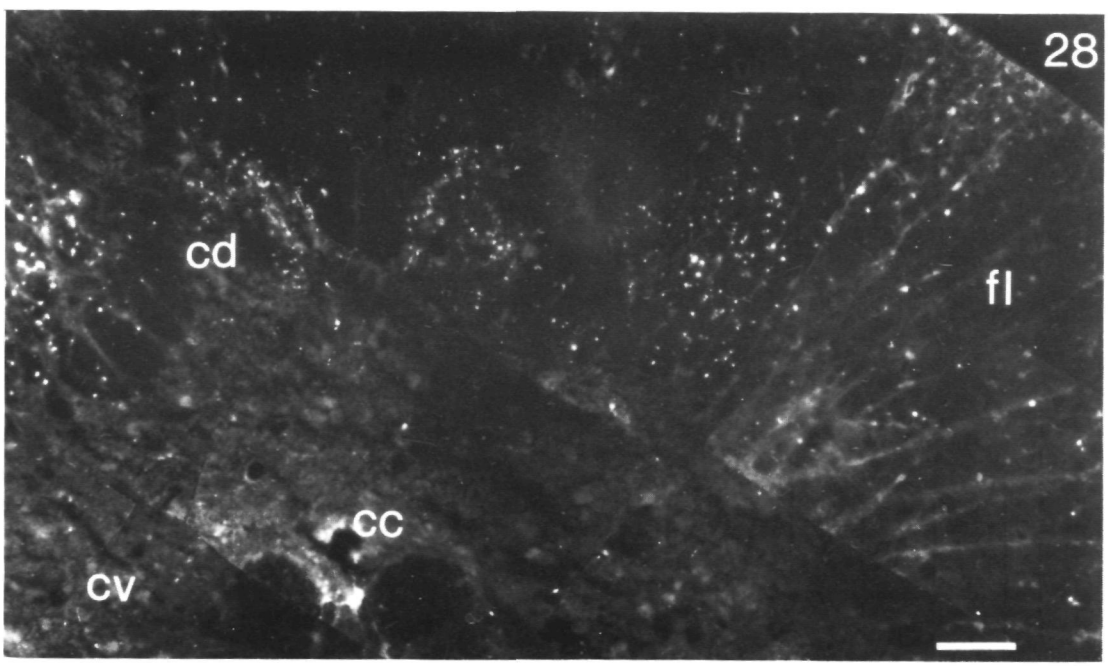
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In the caudal part of the hypothalamus of *Varanus exanthematicus* a large cluster of CA-positive neurons was found in the dorsal part of the lateral hypothalamic area (Figs 1, 12 and 13), probably comparable to group A13[9,48] in mammals

In Table 1 the distribution of CA-containing neurons in the brain stem of vertebrates from lamprey to rat, has been summarized A lateral tegmental cell system is rather constantly present, although its rostral part is apparently absent in bony fishes [105,106] In amphibians these cells so far have only been demonstrated in the salamander *Necturus maculosus*,[17] remaining in a periventricular position The dorsal medullary cell system is a very constant factor in the caudal brain stem, present in all vertebrates studied so far

A cell group, comparable to the locus coeruleus is found in bony fishes,[105,106] amphibians,[64,121,145,161] reptiles,[22,104,108,161] birds[25,36,144] and throughout mammals

So, it appears that the presumably noradrenergic lateral tegmental cell system, the dorsal medullary cell system and the locus coeruleus group, are remarkably constant throughout phylogeny

Clear differences, however, are found as to the presence of CA-positive cells in the mesencephalon In the mesencephalic tegmentum of the lamprey[6] and several bony fishes (e g eel[31] and sunfish[105]) no CA perikarya are observed However, at the mesodiencephalic junction some CA-positive cells are found in the tuberculum posterius (nucleus paratubercularis posterior) of lamprey,[6] and in the nucleus posterior tuberis of sunfish [105] These cells may arise from an anlage, that could be homologous to the one giving rise to the CA mesencephalic neuronal systems in land vertebrates

In the holostean garfish, *Lepisosteus osseus*,[106] apparently for the first time in phylogeny, a small CA-positive cell group near the oculomotor nerve is found Similar observations have been made in salamander[17] and frog [101,104] This cell group becomes more distinct in reptiles and birds, and can be divided into several subsystems, as the substantia nigra (or nucleus tegmenti pedunculopontinus of *Caiman crocodilus* and birds), and the ventral tegmental area

An equivalent for cell group A11 in mammals, has only been described so far in *Varanus exanthematicus* (this study)

The striking similarities between lower vertebrates and mammals in the distribution of CA neurons in the brain stem, do not hold for the hypothalamus In the vertebrate hypothalamus essentially two types of monoaminergic cell bodies can be found [103] (1) small monoaminergic cell bodies located within the hypothalamic periventricular grey, particularly abundant in its ventral portion, (2) medium-sized perikarya at some distance from the ependymal wall of the third ventricle In non-mammalian vertebrates the small neurons outnumber the larger ones, and are mainly confined to highly vascularized ependymal organs such as the preoptic recess organ, particularly abundant in amphibians,[37,101,103,121,123] and the paraventricular organ Furthermore, most of the small periventricular monoaminergic cells in the hypothalamus of non-mammals are in direct contact with the CSF of the third ventricle by means of a short clublike process A mammalian counterpart of the CSF-contacting monoaminergic cells has been reported by Sladek [125]

In the paraventricular organ of various reptiles (*Chrysemys picta*,[103,108] *Lacerta viridis* and *Lacerta muralis*,[7,20,84] and *Caiman crocodilus*[72]) CA-positive, CSF-contacting neurons are found In the turtle *Chrysemys picta* such cells are also present in the preoptic recess organ [103,108] In the paraventricular organ of several lacertilians apart from CA-positive cells, also serotonin-containing neurons have been noticed [70,84,103] The same holds for the paraventricular organ in birds [47,128] In *Varanus exanthematicus*, however, the only hypothalamic CSF-contacting cells observed, are a large amount of serotonergic cells in the paraventricular organ (J G Wolters, H J ten Donkelaar, H W M Steinbusch and A A J Verhofstad, in preparation)

More laterally situated CA cell bodies are found, in restricted number in *Chrysemys picta*,[103,104,108] lateral to the nucleus periventricularis hypothalami, in a position comparable to the large CA cell group in *Varanus exanthematicus* This cell cluster in the dorsal part of the lateral hypothalamic area is probably comparable to group A13 as found in rat [9,48] A non-mammalian homologue for group A12 (the arcuate or infundibular nucleus) may be formed by CA-positive cells around the infundibular recess in the turtle *Chrysemys picta* [103,104,108,109] Surprisingly, in *Varanus exanthematicus* CA-positive CSF-contacting neurons were also found in the spinal cord This is in keeping with very similar findings in the garfish *Lepisosteus osseus*[106] and in the axolotl *Ambystoma mexicanum* [123] In the latter species these neurons have been shown even to be the first CA neurons in the central nervous system to appear during ontogeny [123]

Catecholamine axon pathways in the brain stem and spinal cord

In the present study various CA fiber paths were found which represent the mesostriatal, isthmocortical and medullohypothalamic pathways as described in the turtle *Chrysemys picta* [104,109] In addition, in *Varanus exanthematicus* a periventricular fiber system was found as well as spinal projections

Mesostriatal pathway Rostral to the substantia nigra and ventral tegmental area (Figs 1, 2 and 12) a distinct CA fiber bundle was found, coursing through the lateral hypothalamus, and intermingling with other fibers of the ventral peduncle of the lateral forebrain bundle These observations are in keeping with data in *Chrysemys picta* [104,109] and *Caiman crocodilus* [22] Horseradish peroxidase studies in various reptiles[22,82,102,139] showed ascending projections from the substantia nigra to the striatal part of the telen-

Table 1. Summary of the presence of catecholaminergic cell groups as demonstrated in various vertebrates

			Bony fishes		Amphibians		Reptiles				Mammals	
References	Cyclostomes Lamprey 6, 67, 134	Cartilaginous fishes Dogfish 156	Teleosts Sunfish 73, 105, 143	Holosteans Garfish 106	Salamander 37, 123	Frog 64, 101 104, 121, 161	Turtle 108, 161	Lizard 7, 84,*	Caiman 22	Birds 36, 49, 63, 127, 144	Opossum 31	Rat 35, 57, 99 125, 135
Lateral tegmental cell system												
Medullary part			+	+	+†	−	+	+		+	+	A1, A3‡
Pontine part			−		+†	−	±	+A7(A5?)		+	+	A5, A7
Dorsal medullary cell system			+	+	+	+	+	+		+	+	A2
Locus coeruleus		?	+	+	+	−+§	+	+		+	+	A6
							−	−		−	?	A4
Mesencephalic dopamine cell system	+?		+?	+	+	+−¶	+	+	+	+		A8
								+	+	+		A9
												A10
Midline and periventricular cell system								+?			+	A11
Various diencephalic cell groups					+		+	−		+		A12
							+	+				A13
												A14
Paraventricular organ	+	+	+	+	+	+	+	−+‖	+	+		+**
Preoptic recess organ	+	+	+	+	+	+	+					

*Present study.

†In salamander, these cell groups lie in a periventricular ("embryonic") position [37]

‡The small CA cell group within the dorsal accessory inferior olive of the rat, designated as A3,[35] was not seen in studies making use of immunohistochemical techniques for the localization of CA biosynthetic enzymes [3,135]

§In *Rana catesbiana* CA cells were found medially to the nucleus isthmi,[64] whereas in *Rana temporaria* no isthmic CA cells have been described [101]

¶In *Rana temporaria* CA cells are present in the midbrain,[101] whereas in *Rana catesbiana* no mesencephalic CA cells were found [64]

‖In lacertilians CA cells have been described in the paraventricular organ,[7,84] whereas in *Varanus exanthematicus* no such cells were found (present study)

**In rat subependymal CA cells are found, that may be homologous to morphologically similar cells seen in non-mammalian vertebrates [125]

cephalon by way of the lateral forebrain bundle; in *Varanus exanthematicus*[139] and *Caiman crocodilus*[22] also telencephalic projections of the ventral tegmental area were found. These projections are comparable to the mesostriatal or nigrostriatal dopaminergic projection in mammals (see e.g. Refs[1,2,8,114 and 149]) and the mesolimbic dopaminergic system, arising in the ventral tegmental area.[149]

A mesostriatal projection has also been demonstrated in the salamander *Necturus maculosus*,[17] in the frog *Rana temporaria*[150] and in the pigeon *Columbia livia*.[21,49]

Isthmocortical projection. In turtles[104,110] an isthmocortical pathway has been demonstrated, most likely comparable to the dorsal noradrenergic bundle which innervates the whole mammalian cerebral cortex.[76,149] This fiber bundle is already present very early in ontogeny.[129] Horseradish peroxidase studies in *Varanus exanthematicus*[139] showed that after horseradish peroxidase injections into the basal forebrain some labeled cells were present in the locus coeruleus. Similar observations were made in *Caiman crocodilus*.[22] Therefore, it seems likely that the fiber bundle which could be traced from the level of the locus coeruleus rostralwards (see Figs 1–5, 12, 14, 17 and 20) represents the reptilian homologue of the dorsal noradrenergic bundle (or noradrenergic central tegmental tract[76]). A comparable pathway is also found in the newt *Triturus cristatus*,[35] in the frog *Rana temporaria*,[150] and in birds.[4,144] Even in the bony fishes *Lepisosteus osseus*[106] and *Lepomis gibbosus*[105] a coeruleocortical projection has been made acceptable.

Medullohypothalamic projections. In *Chrysemys picta*, Parent and Poitras[109] found a CA fiber system within the central portion of the medulla, which ascends to the isthmic region and intermingles with the neurons of the locus coeruleus. This fiber system is also found in *Varanus exanthematicus*. The circumscribed area of fine CA varicosities ventral to the nucleus of the solitary tract (Fig. 24) most probably represents the CA medullohypothalamic projection, i.e. the caudal part of the central tegmental tract, originating in the A2 and A1 cell groups.[77,96] The neurons of these CA cell groups have been shown to project predominantly to several hypothalamic nuclei.[19,90,152] In *Varanus exanthematicus* these projections presumably ascend to the hypothalamus with the dorsal noradrenergic bundle. Horseradish peroxidase studies in this lizard[139] showed a well-developed ascending projection from the nucleus of the solitary tract to the diencephalon, which might include an ascending projection from the CA-positive cells in this nucleus.

Periventricular fibers. The dorsal periventricular system[77] is represented in *Varanus exanthematicus* by a more diffuse accumulation of fibers between the ependymal wall of the fourth ventricle, and the nucleus of the solitary tract and the dorsal motor nucleus of the vagus (see Fig. 7). A comparable fiber pattern is found in the medulla oblongata of birds,[36,161] amphibians[37,161] and bony fishes.[105,106,161] In *Varanus exanthematicus* these fibers can be traced rostrally as a separate system along the lateral wall of the fourth ventricle. At the isthmic level it merges with the locus coeruleus fibers. Furthermore, periventricular fibers are present in the hypothalamus of *Varanus exanthematicus*, corresponding to the ventral periventricular system as described in rat.[77]

Descending catecholaminergic projections to the spinal cord. In *Chrysemys picta* a conspicuous descending CA bundle appears to arise from locus coeruleus neurons, i.e. the CA cell group of the isthmus.[109] These fibers form a rather compact bundle that courses along the ventrolateral wall of the brain stem. Horseradish peroxidase studies in various reptiles[95,137,138,141,157,160] showed a distinct coeruleospinal projection. Similar observations were made in *Xenopus laevis*[140] and the pigeon.[25] Locus coeruleus neurons that project to the spinal cord, send their axons predominantly via the ventral funiculus, the ventral part of the lateral funiculus[11,77,97,157] and, at lumbar and sacral levels, via the dorsal part of the lateral funiculus.[152] In rat this coeruleospinal pathway gives rise to the great majority of CA terminals in the spinal cord.[97,152] The innervation of several parasympathetic cell masses, for instance the dorsal motor nucleus of the vagus, and the lateral preganglionic cell column of the sacral spinal cord,[59,154] suggests involvement of the locus coeruleus in the regulation of central parasympathetic outflow, apart from the postulated role in somatosensory[116,122] and motor functions.[52] In the present study the coeruleospinal fibers were only demonstrated in the dorsal part of the lateral funiculus, and CA terminals were only found in the dorsal horn and the dorsal part of lamina X of the spinal grey (Figs 9–11 and 26–28).

Spinal projections from the adjacent part of the lateral tegmental system, i.e. groups A7 and A5, have been demonstrated in rat[5,11,81,120,151,152] as well as in other mammals (e.g. opossum,[32,85,86] cat,[59,70,71,146] rabbit[15] and monkey[154]). In rat the subcoeruleus neurons, that project to the spinal cord via nearly the same funicular trajectory as the locus coeruleus, terminate in comparable regions of the spinal grey, but moreover, in the intermediolateral cell column, whereas innervation of the sacral spinal cord by the A7 neurons is only poor.[152] This termination pattern suggests a role of the subcoeruleus region in the control of sympathetic outflow. A large amount of the CA neurons of the A5 cell group in rabbit, rat and opossum has been shown to project to the spinal cord, making, however, only a minor contribution to the CA innervation of the cord.[15,19,85,120,151,152] Evidence was provided that this pathway, descending via the medial part of the lateral funiculus, terminates bilaterally in the intermediolateral cell column,[11,78,81] thus resembling the subcoeruleospinal projection. This indicates the vasomotor function of the A5 group.[80] Horseradish peroxidase studies in various

reptiles[95 137 138 157 160] suggest that also in the reptilian brain stem cell groups comparable to A7 and A5 are present which project to the spinal cord It should be noted that in reptiles no intermediolateral cell column is present Presumably the cells of origin of preganglionic autonomic fibers are hidden in the central grey (area X) as suggested by Kusuma and ten Donkelaar[68] in turtles

Using a variety of techniques, earlier studies suggested an additional projection of the CA cell groups A1 and A2 to the spinal cord [5 26 70 76 79 89 127] Recent investigations, however, combining horseradish peroxidase and histofluorescence techniques, or making use of antibody to dopamine β-hydroxylase as a retrograde tracer, have made these reports at least doubtful [3 15 19 90 151 152] Possibly the projections of the A1 and A2 CA cell groups to the spinal cord are adrenergic [15 56 118]

From the dorsolateral hypothalamic region, which comprises the large CA cell cluster comparable to the A13 cell group, a direct projection to the spinal cord has been demonstrated in several reptiles (see Ref [141], J G Wolters, unpublished observations with the fluorescent retrograde tracers "Fast Blue" and "Nuclear Yellow") Also in rat,[10 58 174] rabbit,[12] cat[62 70] and monkey[119] a direct hypothalamospinal projection has been demonstrated, which seems to originate mainly from the dorsal and lateral hypothalamic fields,[12 62 70 119 124] comprising the CA cell group A13 Furthermore a catecholaminergic projection from the cell group A11 to the spinal cord has been demonstrated in rat [58 124] The hypothalamospinal projection passes via the ventral and the entire lateral funiculus,[71] and terminates in the intermediolateral cell column of the thoracic spinal cord [78] At least part of this projection is dopaminergic, as shown by a combination of the fluorescent retrograde tracer technique with either immunohistochemistry,[136] or the formaldehyde-induced fluorescence technique [10 174] Also in the pigeon a catecholaminergic hypothalamospinal projection seems likely [25] In various reptiles a projection has been found from the laminar nucleus of the torus semicircularis to the spinal cord [23 138 141] This pathway might also be partly catecholaminergic, regarding the presence of CA cell bodies, assumed to be comparable to the A11 cell group, at its origin

Summarizing, in *Varanus exanthematicus* a number of CA pathways have been demonstrated, that occur in mammals and in several non-mammalian species The data discussed once more stress the phylogenetic constancy of the catecholaminergic innervation of the CNS

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DISTRIBUTION OF SEROTONIN IN THE BRAIN STEM AND SPINAL CORD OF THE LIZARD VARANUS EXANTHEMATICUS: AN IMMUNOHISTOCHEMICAL STUDY

Abstract—The distribution of serotonin-containing nerve cell bodies, fibers and terminals in the lizard *Varanus exanthematicus* was studied with the indirect immunofluorescence technique, using antibodies to serotonin. Most of the serotonin-containing cell bodies were found in the midline, in both of the raphe nuclei, i.e. the nuclei raphes superior and inferior. A considerable number of more laterally shifted serotonergic neurons was found particularly at three levels of the brain stem, viz. in the caudal mesencephalic tegmentum, at the isthmic level, and over a long distance in the medulla oblongata. These laterally situated serotonin-positive neurons were partly found within the confines of the substantia nigra, the nucleus reticularis superior and the lateral part of the nucleus reticularis medius and ventrolateral part of the nucleus reticularis inferior, respectively. No serotonergic cell bodies were found in the spinal cord.

In the brain stem a dense serotonergic innervation was observed in all of the motor nuclei of the cranial nerves, in two layers of the tectum mesencephali, in the nucleus interpeduncularis pars ventralis, the nucleus profundus mesencephali pars rostralis, the periventricular grey, the nucleus parabrachialis, the vestibular nuclear complex, the nucleus descendens nervi trigemini, the nucleus raphes inferior, and parts of the nucleus tractus solitarii.

Descending serotonergic pathways could be traced into the spinal cord via the dorsolateral, ventral and ventromedial funiculi, and were found to innervate mainly three parts of the spinal grey throughout the spinal cord, i.e. the dorsal part of the dorsal horn, the motoneuron area in the ventral horn, and the intermediate zone just lateral to the central canal.

The results obtained in the present study suggest a close resemblance of the organization of the serotonergic system in reptiles and mammals, especially as to the serotonergic innervation of the spinal cord.

Serotonin (5-hydroxytryptamine; 5-HT)-containing neurons have been described systematically for the first time in the rat brain by Dahlström and Fuxe.[26] Since their study the distribution of 5-HT-containing neuronal structures has been investigated in a variety of mammals (opossum,[25] rat,[1,70,84,100,102] cat,[58,91,122] primates[35,47,54,59,73,98] and other species[52,53]) as well as in non-mammalian vertebrates (amphibians,[31,77,78,82,83,97,99] birds,[30,40] bony fishes[5,49,55,85,86,101,107] and cartilaginous fishes[92,93]), including reptiles.[16,17,46,76,81,87,117]

Most of these studies have been carried out with the formaldehyde-induced fluorescence technique[32,33,63] which, however, has the disadvantage of rapid fading of the serotonin fluorescence under the influence of ultraviolet light, while catecholamine fluorescence remains relatively unaffected. In contrast, immunohistochemical techniques, using antibodies to serotonin[104] make it possible to label selectively only 5-HT-containing structures. Thus, the indirect immunofluorescence technique, as used in the present study, forms a much more suitable tool for the demonstration of serotonin-containing neuronal structures.

The aim of the present study is to reveal the presence of serotonergic neurons, nerve fibers and terminals in a reptilian brain stem and spinal cord more accurately than has been possible before. This may provide a scheme for further research on the nature of descending pathways to the spinal cord in reptiles, originating in several brain stem cell groups comparable to nuclei in mammals, known to contain serotonergic neurons.[72,110,115,123]

With the same technique, using antibodies to tyrosine hydroxylase, the distribution of catecholaminergic cell bodies, fibers and terminals has been studied in the lizard *Varanus exanthematicus*.[125]

Preliminary data of this study were presented elsewhere.[124,126]

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Abbreviations: flm, fasciculus longitudinalis medialis; 5-HT, 5-hydroxytryptamine (serotonin); 5-HT$_i$, serotonin immunoreactive; PBS, phosphate-buffered saline.

EXPERIMENTAL PROCEDURES

Experimental animals

Five lizards (*Varanus exanthematicus*) were used, varying in weight from 300 to 550 g, with a total length of 52–70 cm and a snout–vent length of 26–34 cm. The animals were

housed in an air-conditioned room with a fixed temperature and dark–light cycle (10 h, 18°C/14 h, 24 C) Two animals received colchicine (2 mg dissolved in 1 ml 0 9% sodium chloride containing 2% ascorbinic acid) 48 h before being sacrificed (0 08 mg/40 μl in the cisterna magna and 0 4 mg/200 μl i p)

Additional doses were administered 24 h (0 8 mg/400 μl i p) and 2 h (1 2 mg/600 μl i p) before fixation

Preparation of tissues

The animals were anesthetized with a mixture of oxygen, nitrous oxide and halothane, followed by an i v injection of 0 4 ml pentobarbital (Nembutal) Then they were perfused through the heart with ice-cold (4°C) calcium-free Tyrode's solution (Ca^{2+} replaced by Mg^{2+}) for 2 min, immediately followed by 500 ml ice-cold 4% paraformaldehyde dissolved in 0 1 M sodium phosphate buffer, pH 7 3, and post-fixed in the same fixative for 18–24 h The brain stem from the caudal part of the diencephalon up to the spinal cord and pieces taken at four levels of the spinal cord (viz the 6th, 15th, 26th and 38th spinal segments, representing respectively cervical, thoracic, lumbar and tail levels) were frozen with powdered carbon dioxide gas Transversal sections were cut at 10 μm on a cryostat (Dittes, Heidelberg, F R G) Series of sections collected at 22 levels through the brain stem and the four levels of the spinal cord were mounted on glass slides coated with chrome alum gelatin (0 05 g chrome alum and 0 5 g gelatin in 100 ml distilled water) and stored at −70°C pending staining In the brain stem the interval between the first sections of two consecutive series was about 500 μm

Immunofluorescence procedure

Two series of sections were stained according to the indirect immunofluorescence procedure described by Coons and collaborators,[23] employing a rabbit antiserum to bovine serum albumin-coupled serotonin[103 105 118a] for the first, and serum from non-immunized animals for the second series The sections were first rinsed in phosphate-buffered saline (PBS) at room temperature for 30 min Then, they were incubated with the antiserum to serotonin or the non-immune serum, both diluted 1 400 with PBS containing 0 1% Triton X-100 for 18 h at 4°C After rinsing in PBS for 30 min at room temperature, the sections were incubated with fluorescein isothiocyanate-labeled sheep anti-rabbit immunoglobulins (Statens Bakteriologiska Laboratorium, Stockholm, Sweden) diluted 1 16 with PBS, also containing 0 1% Triton X-100 (30 min, room temperature) Finally, they were rinsed in PBS for 30 min at room temperature, mounted in glycerin–PBS (3 1) and stored at −20°C

Evaluation and presentation of results

The sections were examined with a Zeiss Universal microscope, equipped with incident illumination for fluorescence Kodak Tri-X film was used for photomicrography, having exposure times between 15 and 40 s The distribution of immunoreactive cell bodies, fibers and terminals is presented in schematic drawings of cryostat sections stained with cresylechtviolet The density of fluorescent fibers and terminals was classified subjectively into five categories (a) no fluorescence, (b) low density, (c) medium density, (d) high density, (e) very high density

RESULTS

The distribution of 5-HT-immunoreactive (5-HT$_i$) cell bodies, nerve fibers and terminals in the brain stem and spinal cord of the lizard *Varanus exanthematicus* will be described from rostral to caudal, starting at the level of the posterior commissure, down to the caudal medulla oblongata at the level of the obex, followed by four different levels of the spinal cord The terminology used is mainly based on Butler and Northcutt,[19] Cruce and Nieuwenhuys,[24] and ten Donkelaar and Nieuwenhuys [116]

The results are not markedly different between colchicine-treated and non-colchicine-treated lizards, especially not with respect to the distribution and fluorescence intensity of the 5-HT$_i$ fibers The 5-HT$_i$ cell bodies in and medial to the fasciculus longitudinalis medialis (flm) at the isthmic level, however, are only seen after colchicine treatment Besides, in general the fluorescence intensity of 5-HT$_i$ cell bodies is slightly increased after colchicine treatment

Diencephalon

In the diencephalon only one group of 5-HT$_i$ cell bodies is seen, viz the cells in the paraventricular organ, a highly vascularized specialization of the ependymal layer, delineating the sulcus lateralis infundibuli (Fig 14) These neurons are in direct contact with the cerebrospinal fluid of the third ventricle by means of a short club-like process In lateral direction they send very thin predominantly non-varicose fibers, which can be traced over some distance (40–100 μm) In the nucleus periventricularis hypothalami ventral to these neurons, and along the lateral border of the hypothalamus, thick 5-HT$_i$ varicosities are present as well as some coarse non-varicose 5-HT$_i$ fibers The area lateralis hypothalami shows a very dense serotonergic innervation by very fine varicosities and thin varicose fibers

Mesencephalon

In the mesencephalon three groups of 5-HT-positive cell bodies are found, all lying in the caudal midbrain tegmentum (Fig 2) The first group consists of small, oval-shaped perikarya, located in and around the nucleus raphes superior pars lateralis No dendrites or axons can be seen in frontal sections (Figs 2 and 16) A second 5-HT$_i$ cell group, consisting of medium-sized perikarya with processes mainly extending in the horizontal direction, as observed in transversal sections, is found in the ventral tegmentum, lateral to the nucleus interpeduncularis pars ventralis, just caudal to the medial catecholaminergic cell group of the mesencephalon (see Ref 125) This 5-HT$_i$ cell group does not extend beyond the trochlear nucleus (Figs 2 and 16) Continuous with it, the third mesencephalic 5-HT$_i$ cell group extends laterally into the region ventral to the substantia nigra A few cells of this 5-HT$_i$ cell group are lying more dorsally, scattered between the catecholaminergic neurons of the substantia nigra (Figs 2 and 16)

The central and lateral parts of the rostral midbrain tegmentum receive a dense serotonergic innervation (Figs 1 and 15) Especially the cell bodies and dendrites of the nucleus profundus mesencephali pars rostralis are almost completely covered, in a characteristic way, with thin 5-HT$_i$ fibers and varicosities (Figs 1 and 15) In lower magnifications therefore,

this dense innervation pattern may confusingly resemble 5-HT, cell bodies (Fig 15) Throughout the central grey ventral to the lateral recesses of the ventricular system within the mesencephalon, as well as in the nucleus laminaris of the torus semicircularis, fine 5-HT, fibers and varicosities are present (Fig 1) In the nucleus of Edinger–Westphal (not illustrated) and in the nucleus of the fim, fine fluorescent fibers are seen, coursing in ventral direction near the midline (Fig 1) Dorsal to the rostralmost part of the fim fairly thick and brightly fluorescent terminals occur in the nucleus nervi oculomotorii (Figs 2 and 16), surrounding the large motoneuron perikarya In all layers of the tectum mesencephali 5-HT, varicosities are visible, but two layers, i e the broad stratum griseum et fibrosum superficiale, and the narrow stratum griseum centrale, receive a more dense innervation, as shown by intensely fluorescent fibers and terminals In the rostral extension of the ventral tegmental area three different 5-HT, fiber groups are found (Fig 1) The medial confines of this area contain very fine varicosities and thin fibers, continuous with the midline structures Dorsal to this region a well-confined, small bundle of thick cross-cut fibers is present A very dense serotonergic innervation is seen in the ventral part of the ventral tegmental area (Fig 1), and more laterally, in the nucleus opticus tegmenti or nucleus of the basal optic root, whereas the area in between is less densely innervated In the caudal midbrain a dense plexus of fine varicosities is seen in the nucleus centralis of the torus semicircularis, and in the lateralmost part of the brain stem

Isthmus

At the level of the isthmus the highest concentration of serotonergic cells is found in the nucleus raphes superior 25–35 5-HT, perikarya per section, nearly 100% of the total number of raphe cells in this region (Figs 3 and 27) Throughout the rhombencephalon 5-HT, neurons are found in the midline nuclei, i e the nuclei raphes superior and inferior At various levels, however, 5-HT, neurons also occur more laterally, as is the case at the isthmic level Here these laterally shifted 5-HT, neurons are concentrated mainly in two groups in the ventral part of the rostral rhombencephalon (1) a cluster of 5-HT, cells, immediately lateral to the nucleus interpeduncularis pars ventralis, and (2) a cell group continuous with the former, found more laterally in the ventral part of the nucleus reticularis superior pars lateralis (Figs 3 and 17) The processes of these cells are oriented parallel to the ventral surface of the brain stem Serotonin-immunoreactive cell bodies are also found interspersed between the medial border of the fim and the ventricle Few 5-HT, cells occur in the medial part of the central grey, in the locus coeruleus, and occasionally a 5-HT, neuron is found lateral to the cerebellar nuclei (Fig 3)

Very fine varicosities are present throughout the rhombencephalon They are tightly packed in the nucleus parabrachialis, in the periventricular grey, and dorsal to the nucleus raphes superior (Fig 3) Very fine varicose fibers, coursing in the transversal plane, are seen in the molecular layer of the cerebellum and in the nucleus of the lemniscus lateralis In the granular layer of the cerebellum and in the cerebellar nuclei fine varicosities are found Non-varicose as well as varicose fibers are seen along the lateral wall of the brain stem, lateral to the brachium conjunctivum (Fig 3)

Medulla oblongata

In the medulla oblongata the bulk of serotonergic neurons occurs in the nucleus raphes inferior (Figs 4–8, 19, 20 and 22) It should be noted, that in the nucleus raphes inferior predominantly large, partly very large, polygonal perikarya are found, with sometimes fairly thick processes traceable over considerable distances into the adjoining reticular core (Fig 19) At the level of the eighth cranial nerve, however, only a very limited number of 5-HT, cell bodies (one to four per section) is present in the midline, i e in the ventralmost part of the nucleus raphes inferior Here, the majority of 5-HT, cells is concentrated in a lateral group, located just ventral to the nucleus descendens nervi trigemini and the motor nucleus of the facial nerve (Fig 5) These laterally situated cells are mainly of the same type as seen at the isthmic and midbrain levels, i e medium-sized, oval-shaped cells, with thin processes More caudally this lateral cell cluster gradually shifts medialwards (Figs 6 and 7) and finally merges with the numerous 5-HT, cells present in the nucleus raphes inferior At the level of the obex, the great majority of serotonergic neurons is found in the midline, whereas some scattered 5-HT, cells are present in the ventralmost part of the nucleus reticularis inferior, at some distance from the nucleus raphes inferior At the level of the entrance of the trigeminal nerve, only very few laterally situated 5-HT, perikarya are found (Figs 4 and 20)

In the rostral part of the rhombencephalon varicose fibers are found throughout the nucleus reticularis medius, without any preferential direction in its central part, but, closer to the ventral and ventrolateral brain stem surface more or less perpendicular to it (Figs 4 and 20) Serotonergic fibers cross the midline dorsal to the nucleus raphes superior, passing through the middle part of the fim (Fig 4) In the middle and ventral part of the fim fluorescent varicose structures probably represent cross-sectioned ascending and descending serotonergic projections In the dorsal and ventral motor nuclei of the trigeminal nerve, a great density of 5-HT, varicosities is found, surrounding the motoneurons (Figs 4, 18 and 20), a similar feature as seen in the nucleus nervi oculomotorii (Fig 16) Many varicosities are found in the nucleus princeps nervi trigemini, in the peri-

ventricular grey, the raphe and the adjacent reticular formation (Figs 4 and 20). The nucleus descendens nervi trigemini receives a dense serotonergic innervation, especially its dorsal and lateral part, by very fine varicosities (Figs 4–9, 20 and 21). At the level of the entrance of the eighth nerve, however, the ventral part of this nucleus shows numerous fairly thick varicosities. In the vestibular nuclear complex many fine 5-HT$_1$ varicose fibers are present, arranged in a typical and distinct way (Figs 4–6, 20, 21 and 24). The nucleus of the abducens nerve receives a very dense serotonergic innervation as shown by the numerous terminals covering the surface of its large motoneurons (Figs 5, 21 and 25). The same holds for the nucleus nervi facialis (Fig. 30). The periventricular grey throughout the medulla oblongata, the adjacent medial part of the nucleus of the solitary tract and the dorsal motor nucleus of the vagus receive a rich but diffuse serotonergic innervation (Figs 6 9 and 21–23). A thin strip at the lateral border of transverse sections of the entire medulla oblongata (Figs 5–9), as was seen earlier at the isthmic (Fig. 3) and caudal mesencephalic (Fig. 2) levels, shows a similar picture. In the nucleus of the hypoglossal nerve, 5-HT$_1$ varicosities surround the contours of its motoneurons (Figs 7 9, 22 and 23), suggesting a similar innervation as seen in the nucleus nervi abducentis and the nucleus nervi oculomotorii. Many parts of the ventral and lateral reticular formation show a diffuse but dense serotonergic innervation; in addition many fine varicose fibers occur, more or less oriented in the transversal plane. Close to the brain stem surface these fibers are directed perpendicularly to it, more centrally they course parallel to the brain stem surface.

Spinal cord

The serotonergic innervation of the spinal cord in the lizard *Varanus exanthematicus*, is much more impressive than that by catecholamines.[125] Throughout the spinal cord three parts of the spinal gray are particularly innervated: (1) the dorsal horn, where terminal structures are most numerous in the dorsalmost layers, (2) the intermediate zone, more in particular lateral to the central canal, and (3) the ventral horn, where 5-HT$_1$ terminal structures surround the motoneurons (Figs 28 and 29), a similar feature as observed in several motor nuclei in the brain stem (Figs 10–13, 28 and 29). A strict localization in specific areas as distinguished by Kusuma *et al.*[57] is not found. Irrespective of slight differences in distribution of immunoreactivity between the left and right sides of the same spinal segment (Figs 10–13 and 28), there is a close resemblance between both sides, not only regarding the distribution of terminal structures, but also with respect to the funicular trajectory of descending serotonergic projections. At all levels cross-sectioned fibers are seen particularly in the dorsolateral part of the lateral funiculus, and in the ventral and medial parts of the ventral funiculus (Figs 28 and 29). The density and intensity of these 5-HT$_1$ cross-cut nerve fibers, however, is not the same at all levels (Figs 10–13, 28 and 29). In tail segments, for instance, these fibers are especially numerous, and remarkably thicker than elsewhere in the spinal chord (Fig. 28).

DISCUSSION

Distribution of serotonin-containing neurons in the brain stem and spinal cord

The use of the indirect immunofluorescence technique has provided a much more complete picture of the serotonergic innervation of the brain stem and spinal cord in a reptile than was known before.[16 17 76,81 82 87]

Prior to a discussion of our results a brief outline of the 5-HT cell groups in the mammalian brain stem will be given The following cell groups can be distinguished in the mammalian brain stem (e.g. in opossum,[25] rat,[26 70 100 102] cat[58 122] and monkey[34 35 98])

(1) The caudal medullary 5-HT cell groups B_1, B_2 and B_3, as originally described by Dahlström and Fuxe,[26] which correspond largely to the nucleus raphes pallidus, nucleus raphes obscurus and nucleus raphes magnus, respectively;

(2) The dorsal periventricular 5-HT cell groups B_4 and B_6. B_4 a small cell group situated in the vicinity of the lateral part of the medial vestibular nucleus close to the midline, B_6 is considered either as a separate entity or as a caudal extension of the nucleus raphes dorsalis,

(3) The pontine tegmental 5-HT cells, i.e. B_5, a rather small group, located mainly in the intermediate part of the nucleus raphes pontis at the level of the trigeminal motor nucleus;

(4) The midbrain 5-HT cell groups B_7, B_8 and B_9 of Dahlstrom and Fuxe.[26] These groups are located in the nucleus raphes dorsalis (situated just ventral to the periaqueductal grey), the nucleus centralis superior and lateral to the nucleus centralis superior, respectively

Furthermore, recently a group of 5-HT$_1$ neurons has been observed in the rat hypothalamus, viz. in the nucleus dorsomedialis hypothalami.[6 21 37 102 106] Since these cells, however, only were found following pharmacological manipulations, they might be serotonin-accumulating rather than serotonin-containing cells. In the cat hypothalamus, however, 5-HT-containing cell bodies have been described, using the Falck–Hillarp fluorescence histochemical method, with and without any further pharmacological treatment [75] These neurons were situated in the lateralmost part of the lateral hypothalamic area.

Data in rat[8 12,65 74] indicate that the medullary cell groups B_1–B_3 account for some 30% of the total serotonergic population in the brain stem, the majority of 5-HT$_1$ neurons lying in the midbrain cell groups. Although most of the serotonergic perikarya are located in the raphe nuclei, a significant number (in cat: 22.5%[122]) is found outside the raphe nuclei at all brain stem levels. Particularly in cat,[58,122] but also in rat[100 102] a distinct lateral shifting of 5-HT$_1$ cell bodies can be observed; in opossum,[25] however, 5-HT$_1$ cells are mainly restricted to the midline

In the present study in the lizard *Varanus exanthematicus* a rather similar distribution pattern 5-HT$_1$ cell bodies in the brain stem is observed as is found in mammals. In reptiles only two raphe nuclei can be distinguished,[24,116] i.e. the rostral, mainly small-celled nucleus raphes superior, and the large caudal nucleus raphes inferior, containing medium-sized to very

large cells (see Fig 30) The nucleus raphes superior, composed of a medial and lateral part (Figs 2, 3 and 30) extends from the caudal midbrain to the level of the trigeminal nerve root, the nucleus raphes inferior from that level to the first spinal segment Of the various cell types in the nucleus raphes inferior, the very large ones are mainly concentrated (Fig 30) in an area at the level of the ventromedial vestibular nucleus (Fig 6) and just caudal to that (Fig 7) Except for a sparse 5-HT$_1$ cell between the flm (Fig 7) all medullary 5-HT$_1$ cell bodies in *Varanus exanthematicus* are found ventral to the flm with a distinct lateral shifting of perikarya as also observed in rat[100 102] and cat [122] The major portion of these lateral cells is found rostrally (Figs 5 and 6) As regards their relative position in the caudal brain stem, the 5-HT$_1$ cell bodies indicated in the inferior raphe nucleus in Figs 8 and 9 presumably are comparable to those in the nucleus raphes pallidus in mammals, those in Figs 6 and 7 to the nucleus raphes magnus No apparent homologue of the 5-HT$_1$ cells in the mammalian nucleus raphes obscurus (mainly situated between the flm) was observed The 5-HT$_1$ raphe cells in Fig 4 at the level of the motor nucleus of the trigeminal nerve, and also directly rostral to this level, show resemblance to the mammalian nucleus raphes pontis

At the level of the cerebellar peduncle (Fig 3) a distinct cluster of 5-HT$_1$ perikarya appears in the medial part of the nucleus raphes superior At this level also the extensive lateral shifting of 5-HT$_1$ neurons should be stressed More rostrally (Fig 2) 5-HT$_1$ cell bodies are found in the lateral parts of the nucleus raphes superior, and again in considerable numbers more laterally They are situated immediately ventral to the caudal part of the substantia nigra (Fig 16) Here 5-HT- and dopamine-containing neurons[81 125] to a certain degree intermingle

In the caudal midbrain no 5-HT$_1$ cell group was found in the area just dorsal to the flm, i e in a position comparable to that of the nucleus raphes dorsalis in mammals However, slightly more caudally (Fig 3) a group of 5-HT$_1$ neurons is found which might represent the caudal part of the mammalian nucleus raphes dorsalis (B_6) As regards their relative position within the caudal midbrain and isthmic region the 5-HT$_1$ cells in *Varanus exanthematicus* are comparable to those in the nucleus centralis superior (B_8), the lateral extension to B_9 A primordium of the nucleus raphes dorsalis, however, might be hidden in the nucleus raphes superior

In other reptiles studied (*Chrysemys picta*,[76 80 81 87] *Gekko gecko*[46]) 5-HT neurons are apparently more restricted to the midline than in *Varanus exanthematicus*, although also a lateral shifting of 5-HT$_1$ neurons was observed in the caudal midbrain In the turtle *Clemmys japonica*[117] a lateral shifting of 5-HT$_1$ cell bodies is also present at certain levels of the medulla oblongata

Such a lateral shifting of 5-HT$_1$ neurons can also be observed in the caudal part of the midbrain of birds[30 40] and throughout the rhombencephalon of the stingray *Dasyatis sabina* [93] In amphibians[31 97 99] and in bony fishes,[85 86] however, 5-HT cells occur almost exclusively in the midline raphe nuclei Only in the frog *Rana temporaria*[77] more laterally situated 5-HT neurons have been found in the midbrain It should also be noted that already in the primitive vertebrate as the lamprey, *Lampetra fluviatilis*, seven distinct 5-HT$_1$ cell groups have been disclosed,[5 101] most of which, however, are not situated in the midline, but close to the fourth ventricle, dorsolaterally to the flm This location resembles early stages in the development of the raphe nuclei in mammals The raphe nuclei arise from paired anlagen near the ventricle Raphe cells (including serotonergic elements) migrate in ventral direction and subsequently medialward to reach the raphe region [60 62 95 102 119]

It should be noted, that although most of the neurons present in the raphe nuclei of *Varanus exanthematicus* contain serotonin (nearly 100% in the nucleus raphes superior, and about 70–90% in the nucleus raphes inferior), some raphe cells in reptiles are non-serotonergic Whether these cells contain the same putative neurotransmitters as have been shown in mammals[15 42] remains to be investigated In the lizards *Anolis carolinensis*[71] and *Varanus exanthematicus* (J G Wolters, H J ten Donkelaar and A A J Verhofstad, in preparation) no enkephalinergic neurons have been found so far in the raphe nuclei

In addition to the serotonergic brain stem cell groups in lower vertebrates as amphibians,[29 30] reptiles (see Refs 16, 66, and the present study) and birds[80 82 83] serotonergic cerebrospinal fluid-contacting neurons are found in the paraventricular organ, i e an ependymal specialization of the hypothalamus The paraventricular organ has been suggested to play an important role in reproductive function [29 66 80] Its monoamine content depends e g on seasonal factors and temperature [29 66] The absence of serotonin in the paraventricular organ in certain species (the turtle *Chrysemys picta*[80 81 87 88] and the axolotl *Ambystoma mexicanum*[97] should therefore be interpreted with caution due to temporary changes in biological circumstances serotonin concentration might decrease beyond the detection level of the used techniques In mammals also serotonin-containing cell bodies have been disclosed in the hypothalamus [6 21 37 50 90 102 106] Although some of them are located in the vicinity of the ependymal layer, no cerebrospinal fluid-contacting processes have been described

These neurons presumably fulfill only an intrinsic role within the medial hypothalamus and possibly modulate the descending dopaminergic fibers arising in the zona incerta to the eminentia mediana [6 36] Moreover, it should be noted that these 5-HT$_1$ neurons could be detected only by autoradiography or after pharmacological manipulation During ontogenesis these neurons may have lost their capacity to

synthetize serotonin [106 120] It should also be noted that in mammals 5-HT, tanycytes are found in the region of the eminentia mediana [102] In addition, mammals have an extensive supraependymal serotonergic plexus, arising in the nucleus raphes dorsalis and nucleus centralis superior So, reptiles and mammals have in common neuronal elements which are in direct contact with the cerebrospinal fluid, but their organization is different

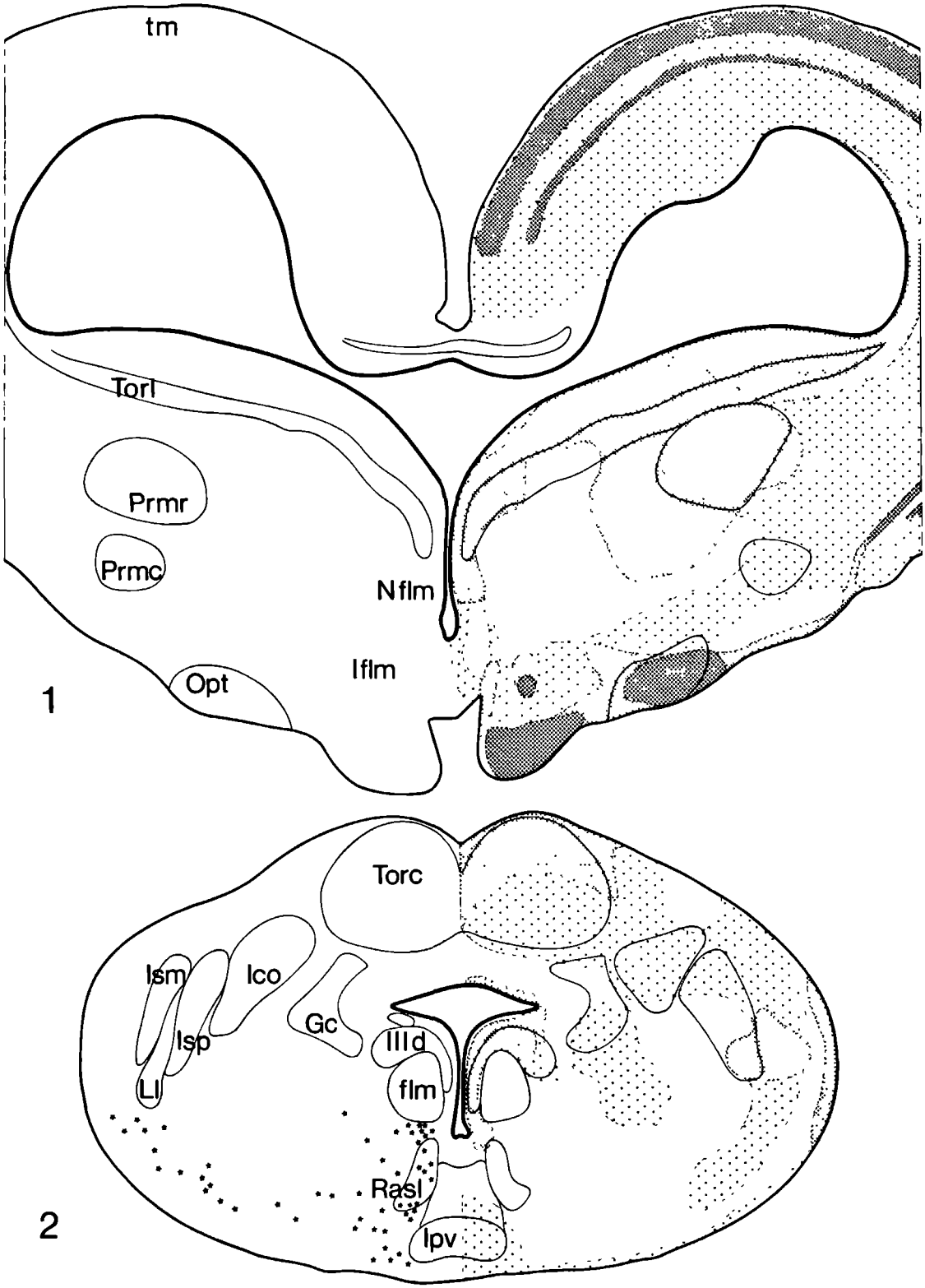
Finally, serotonergic neurons are present throughout the spinal cord in the stingray *Dasyatis sabina* [92,93] Most of them are found in the midline, ventral to the central canal, with vertically oriented processes Similar findings have been reported in monkey [59] In opossum 5-HT, neurons are present in the grey matter, close to the central canal, and in the white matter of the ventromedial funiculus during development In the adult animal, however, such cells cannot be demonstrated anymore [28] In the lizard *Gekko gecko*[46] a few 5-HT, cell bodies were also found ventral to the central canal In *Varanus exanthematicus*, however, no 5-HT, neurons were found in the spinal cord, but in contrast catecholamine-containing neurons were present in a comparable position [125] Similar observations have been made in the axolotl [97]

These differences may be related to a matter of staining specificity The serotonin antibody shows to

Abbreviations used in figures

Amb	nucleus ambiguus
cb	cerebellum
Cerl	nucleus cerebellaris lateralis
Cerm	nucleus cerebellaris medialis
Coa	nucleus cochlearis angularis
Codm	nucleus cochlearis dorsalis magnocellularis
cp	commissura posterior
Cs	nucleus centralis superior
Fl	nucleus funiculi lateralis
flm	fasciculus longitudinalis medialis
Fun	nucleus funiculi dorsalis
Gc	griseum centrale
gl	lamina granularis cerebelli
Ico	nucleus intercollicularis
Iflm	nucleus interstitialis of the flm
IIId	nucleus nervi oculomotorii, pars dorsalis
IIIi	nucleus nervi oculomotorii, pars intermedia
Ipv	nucleus interpeduncularis, pars ventralis
Ism	nucleus isthmi, pars magnocellularis
Isp	nucleus isthmi, pars parvocellularis
Lc	locus coeruleus
Li	nucleus linearis intermedius
Ll	nucleus lemnisci lateralis
Lr	nucleus linearis rostralis
ml	lamina molecularis cerebelli
Nflm	nucleus of the flm
nIII	nucleus oculomotorius
nV	nervus trigeminus
nVI	nervus abducens
nVIII	nervus vestibulocochlearis
Ols	nucleus olivaris superior
Opt	nucleus opticus tegmenti (= nucleus of the basal optic root)
Pb	nucleus parabrachialis
Prmc	nucleus profundus mesencephali, pars caudalis
Prmr	nucleus profundus mesencephali, pars rostralis
Pvo	paraventricular organ
Rai	nucleus raphes inferior
Ram	nucleus raphes magnus
Rasl	nucleus raphes superior pars lateralis
Rasm	nucleus raphes superior, pars medialis
Rd	nucleus raphes dorsalis
Ri	nucleus reticularis inferior
Rm	nucleus reticularis medius
Ro	nucleus raphes obscurus
Rpa	nucleus raphes pallidus
Rpo	nucleus raphes pontis
Rs	nucleus reticularis superior
Rsl	nucleus reticularis superior, pars lateralis
Sn	substantia nigra
Sol	nucleus tractus solitarii
tm	tectum mesencephali
Torc	nucleus centralis, torus semicircularis
Torl	nucleus laminaris, torus semicircularis
Vds	nucleus descendens nervi trigemini
Vedl	nucleus vestibularis dorsolateralis
Veds	nucleus vestibularis descendens
Vevl	nucleus vestibularis ventrolateralis
Vevm	nucleus vestibularis ventromedialis
VI	nucleus nervi abducentis
VII	nucleus nervi facialis
vIII	ventriculus tertius
vIV	ventriculus quartus
Vmd	nucleus motorius nervi trigemini, pars dorsalis
Vmv	nucleus motorius nervi trigemini, pars ventralis
Vnc	vestibular nuclear complex
Vpr	nucleus princeps nervi trigemini
XII	nucleus nervi hypoglossi
Xmd	nucleus motorius dorsalis nervi vagi

Figs 1–13 Schematic representation of 5-HT immunoreactivity as observed in representative transversal sections of the brain stem and spinal cord of colchine-treated lizards At the right, the density of immunoreactive fibers and varicosities is indicated, subjectively classified into five categories no fluorescence, low density, medium density, high density and very high density At the left and in the midline asterisks indicate immunofluorescent cell bodies in each section The numbers 6, 15, 26 and 38 in Figs 10–13 refer to the respective levels of the pictured spinal segments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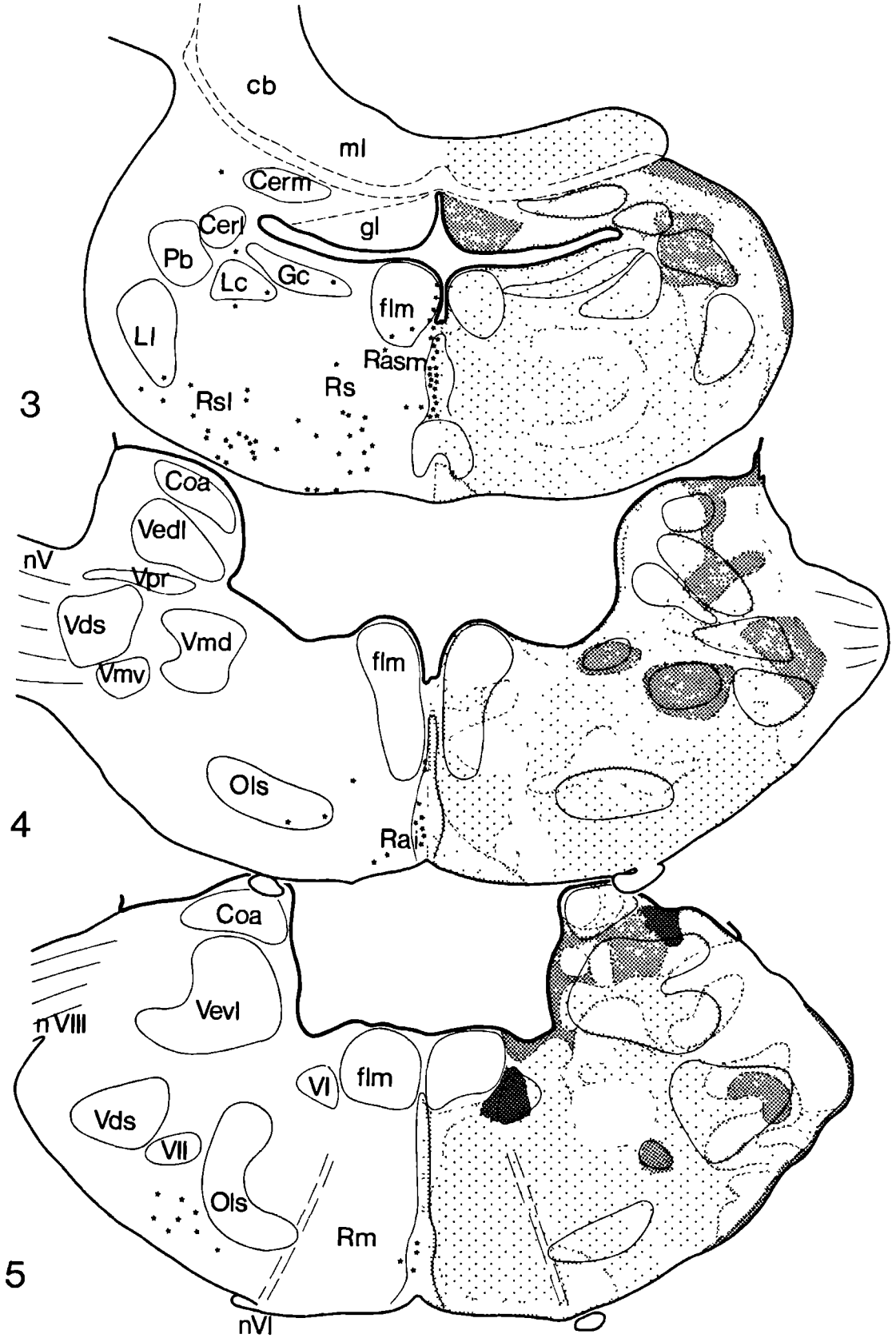
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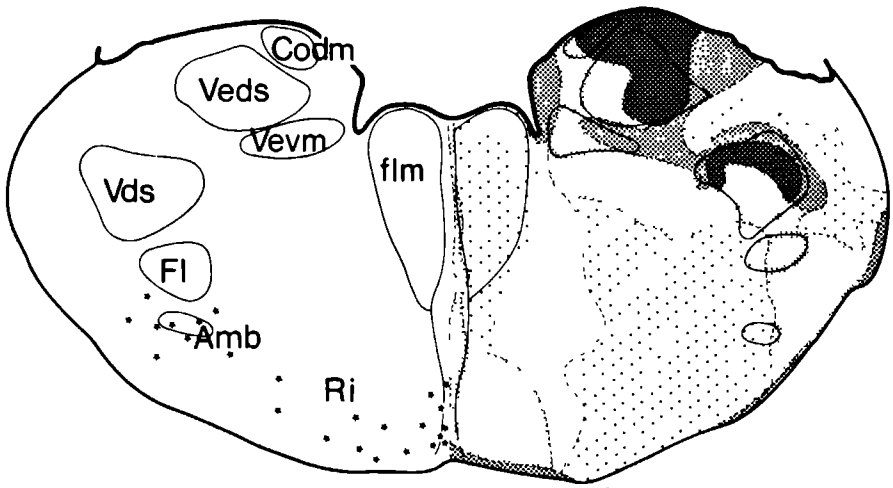
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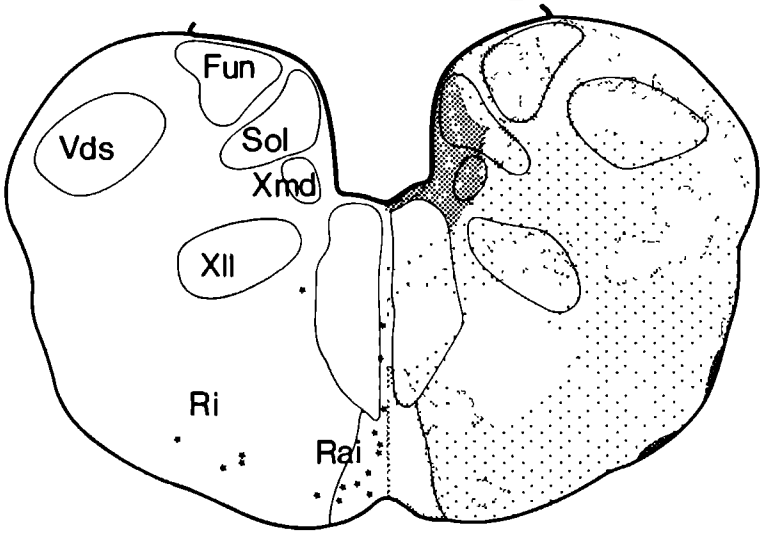
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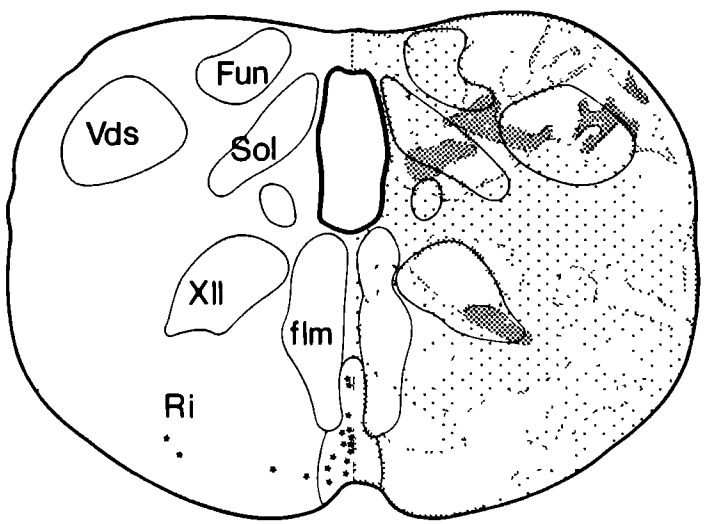
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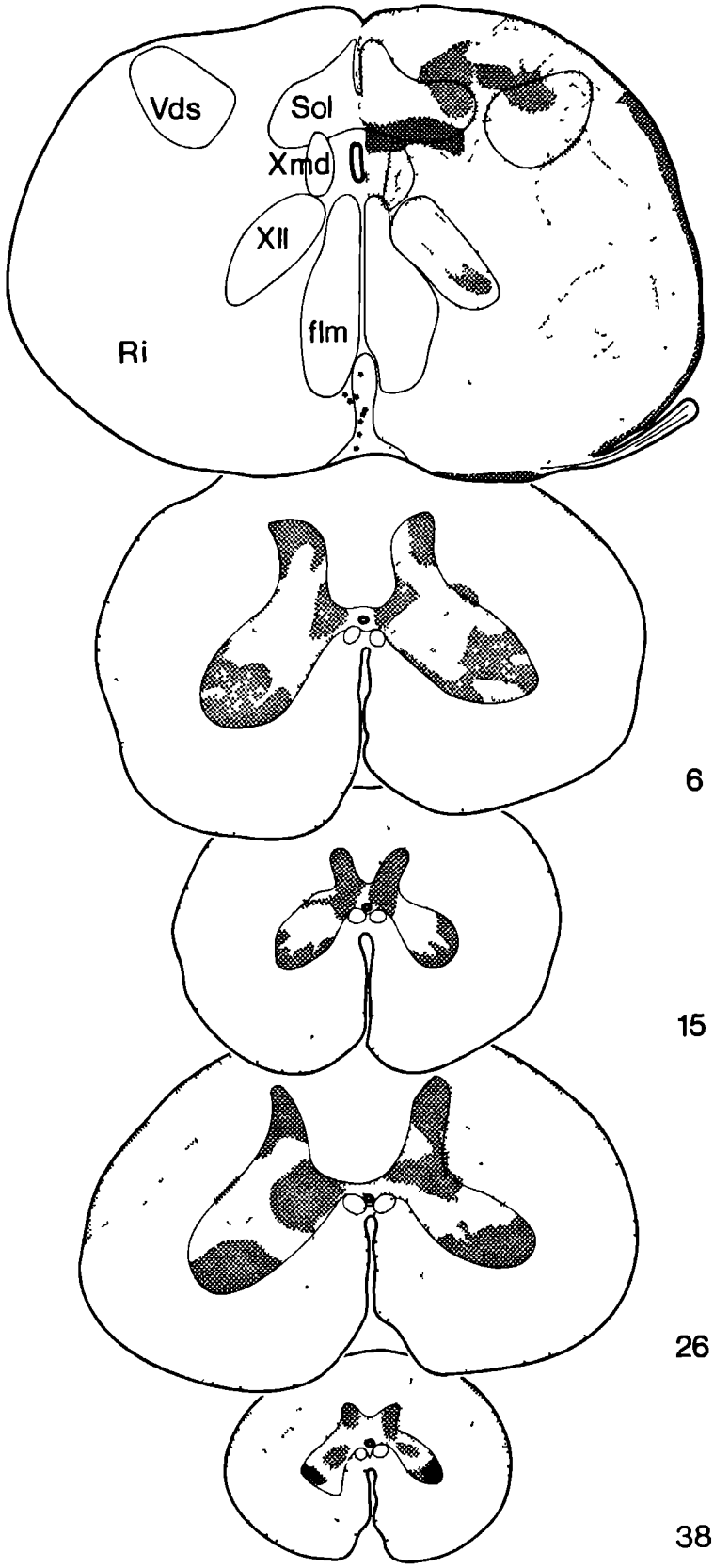
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Fig 14 Hypothalamus Serotonin-immunoreactive neurons in the paraventricular organ Medially these cells are in direct contact with the cerebrospinal fluid, laterally they send very thin processes into the neighboring nucleus periventricularis hypothalami, where terminal-like structures can also be observed (Bar 60 μm)

Fig 15 Rostral mesencephalon Neurons in the nucleus profundus mesencephali pars rostralis The perikarya and processes are completely covered with thin 5-HT$_1$ fibers and varicosities (left = lateral) In the upper right corner, the fibers of the crossed and uncrossed tractus tectobulbares can be seen Dorsomedially to these tracts, the medium density 5-HT innervation of the laminar nucleus of the torus semicircularis and adjacent central grey can be observed (Bar 150 μm)

Fig 16 Caudal mesencephalon General view of the three midbrain 5-HT$_1$ cell groups (a) in and around the nucleus raphes superior, pars lateralis, (b) in the ventral tegmental area, and (c) laterally extending 5-HT$_1$ neurons ventral and ventrolateral to the substantia nigra, as well as a few more dorsally located 5-HT$_1$ cells within the confines of the substantia nigra In the nucleus nervi oculomotorii a dense plexus of 5-HT$_1$ terminal structures is seen, covering the motoneurones Furthermore, the lateralmost part of the brain stem is densely innervated (Bar 300 μm)

Fig 17 Isthmic tegmentum Laterally shifted 5-HT$_1$ neurons, extending in the nucleus reticularis superior, pars lateralis Arrow indicates the nucleus raphes superior, lying just outside the picture Note the numerous 5-HT$_1$ fibers and varicosities all over the isthmic tegmentum (Bar 150 μm)

Fig 18 Rostral rhombencephalon Nucleus motorius nervi trigemini, pars ventralis, detail A dense plexus of very thin 5-HT$_1$ varicose fibers is present throughout the nucleus Note, that the varicosities differ in size, ranging from very fine to thick, and that the thick ones mainly surround the contours of the motoneurones (Bar 45 μm)

Fig 19 Rostral rhombencephalon Large 5-HT$_1$ neurons in the rostralmost part of the nucleus raphes inferior The fairly thick processes of these cells course mainly in a horizontal direction into the adjoining reticular formation These 5-HT$_1$ varicose fibers are present in as well as next to the raphe nucleus (Bar 45 μm)

Fig 20 Rostral rhombencephalon General view of 5-HT$_1$ immunoreactivity at the level of the entrance of the trigeminal nerve Serotonin-immunoreactive cell bodies are only present in the nucleus raphes inferior The nucleus motorius and princeps nervi trigemini are densely innervated At the ventrolateral border of the brain stem a distinct area is densely innervated by 5-HT$_1$ fibers, organized more or less perpendicular to the brain stem surface Horizontally coursing 5-HT$_1$ fibers, just ventral to the fim, seem to interconnect the nucleus raphes superior and more lateral brain stem regions Furthermore, 5-HT$_1$ varicosities are present in the nucleus vestibularis dorsolateralis, and throughout the reticular core (Bar 300 μm)

Fig 21 Rhombencephalon, middle part A dense 5-HT$_1$ plexus is seen in the dorsolateral part of the vestibular nuclear complex, as well as in the dorsal part of the nucleus descendens nervi trigemini The nucleus nervi abducentis receives a very dense 5-HT$_1$ innervation, shown by numerous varicosities surrounding the motoneurons Note furthermore the presence of 5-HT$_1$ varicose fibers in the subependymal layer, and their typical course within the nucleus vestibularis ventrolateralis (Bar 150 μm)

Fig 22 Medulla oblongata General view of the 5-HT immunoreactivity in its medial part 5-HT$_1$ perikarya in the nucleus raphes inferior, and 5-HT$_1$ varicose structures in the ventral part of this nucleus and in the adjacent reticular formation A dense plexus of varicose fibers is present in the nucleus tractus solitarii and the nucleus motorius dorsalis nervi vagi, as well as in the nucleus nervi hypoglossi (Bar 300 μm)

Fig 23 Medulla oblongata, detail of Fig 22 A diffuse, but dense plexus of 5-HT$_1$ varicose fibers in the nucleus tractus solitarii and the nucleus motorius dorsalis nervi vagi In the nucleus nervi hypoglossi, 5-HT$_1$ varicosities surround the contours of the motoneurons (Bar 150 μm)

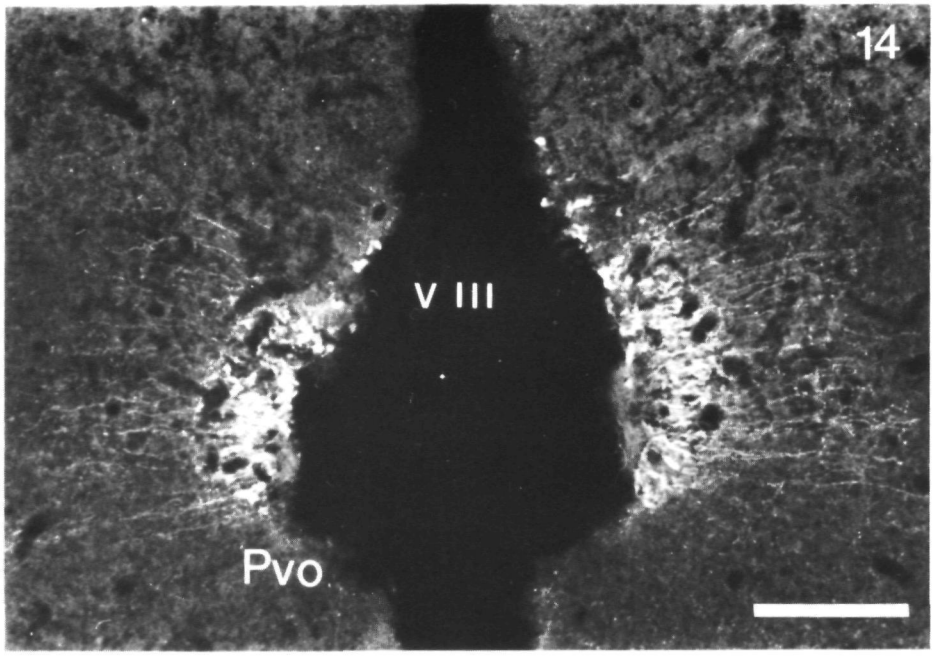
Fig 24 Rhombencephalon, middle part Fine and very fine 5-HT$_1$ varicose fibers, innervating the vestibular nuclear complex (Bar 45 μm)

Fig 25 Rhombencephalon, middle part Fairly thick to thick 5-HT$_1$ varicosities, covering the surface of the motoneurones in the nucleus nervi abducentis Note the presence of very thin 5-HT$_1$ varicose and non-varicose fibers (Bar 45 μm)

Fig 26 A tail level of the spinal cord, detail of Fig 28 Very fine to thick 5-HT$_1$ varicosities in the motoneuron area of the ventral horn Sparsely very thin varicose as well as non-varicose fibers are seen (Bar 30 μm)

Fig 27 Rhombencephalon, middle part Serotonin-immunoreactive neurons in the caudalmost part of the nucleus raphes superior Thick caliber processes of these cells as well as 5-HT$_1$ varicose and non-varicose fibers in this nucleus are arranged in vertical direction Very fine to fairly thick 5-HT$_1$ varicosities indicate a 5-HT$_1$ innervation of the nucleus raphes superior of medium density (Bar 45 μm)

Fig 28 A tail level of the spinal cord General view of the 5-HT immunoreactivity, varicosities occur in the dorsal horn, the intermediate zone, and the ventral horn of the spinal grey Cross-sectioned 5-HT$_1$ fibers are present in both the lateral and ventral funiculi (Bar 150 μm)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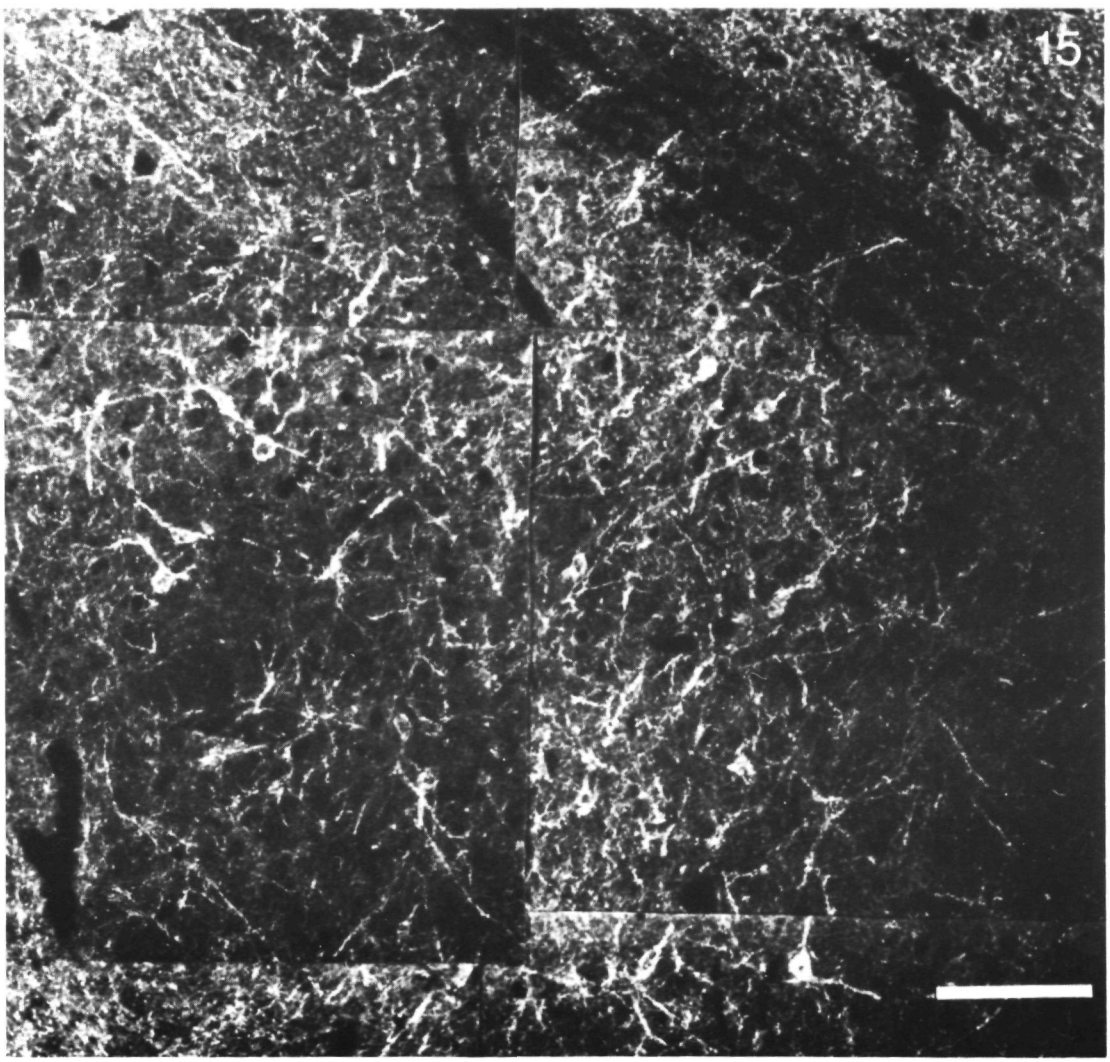
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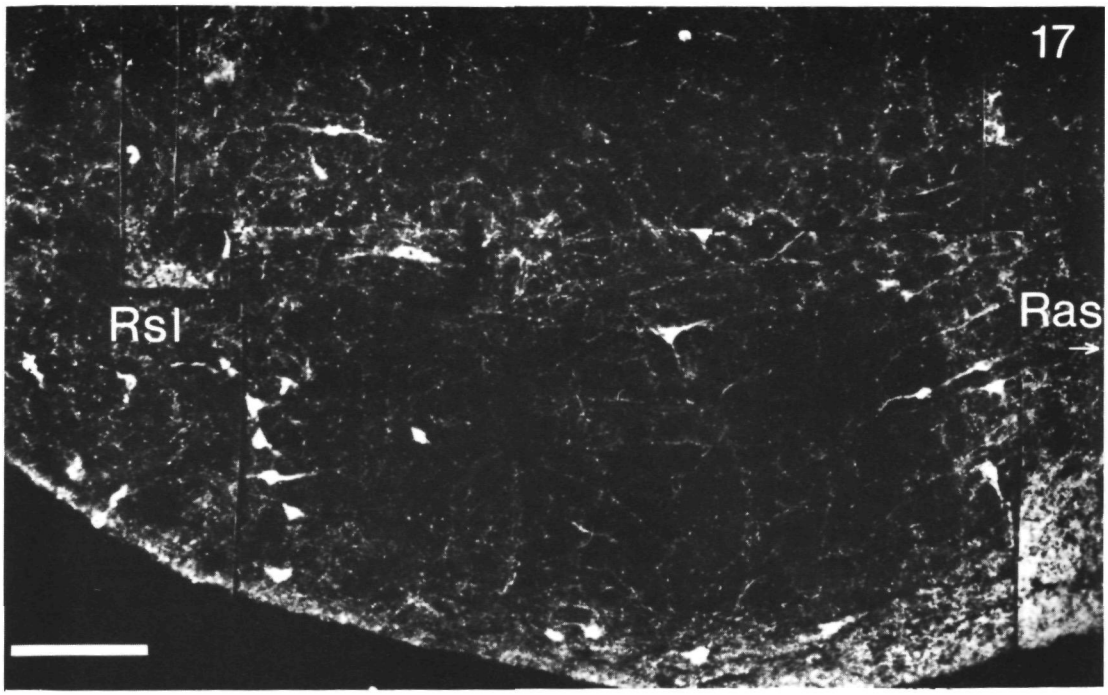
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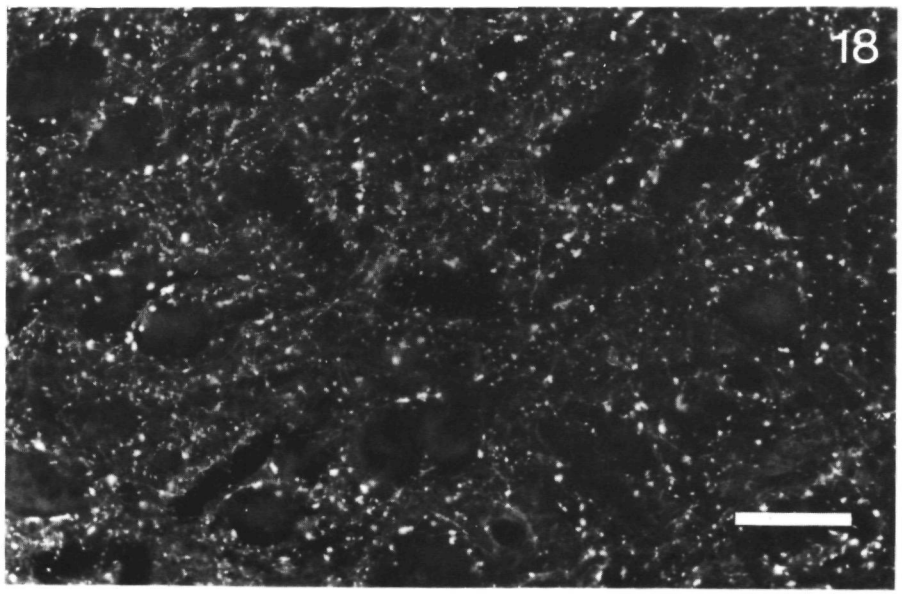
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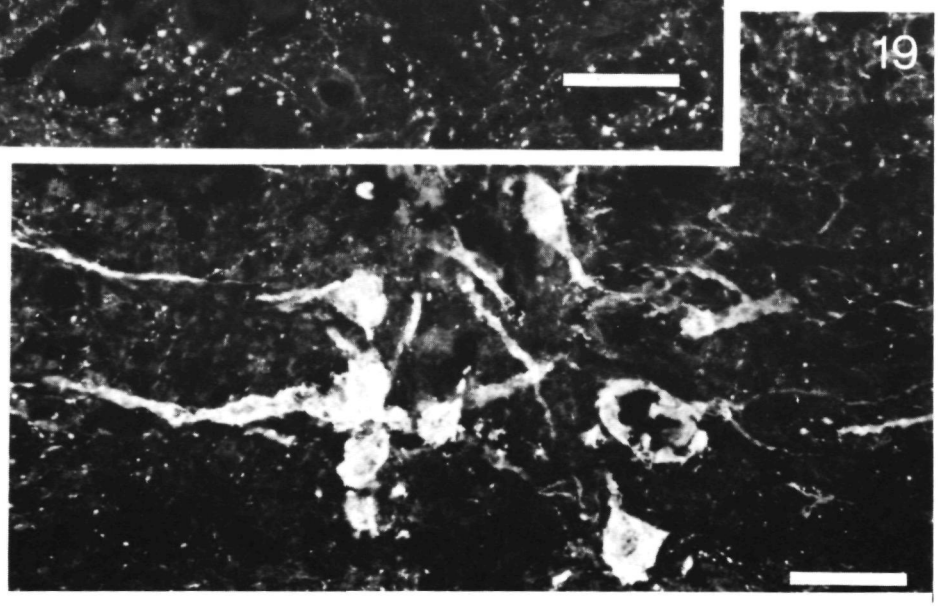
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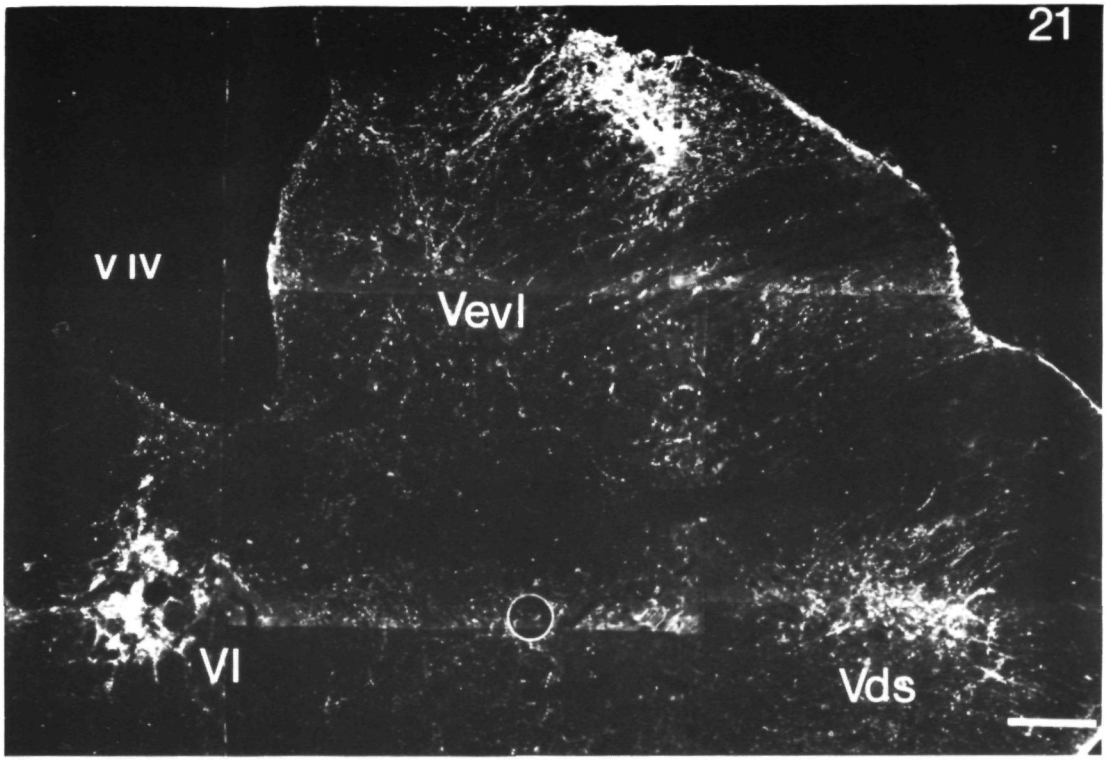
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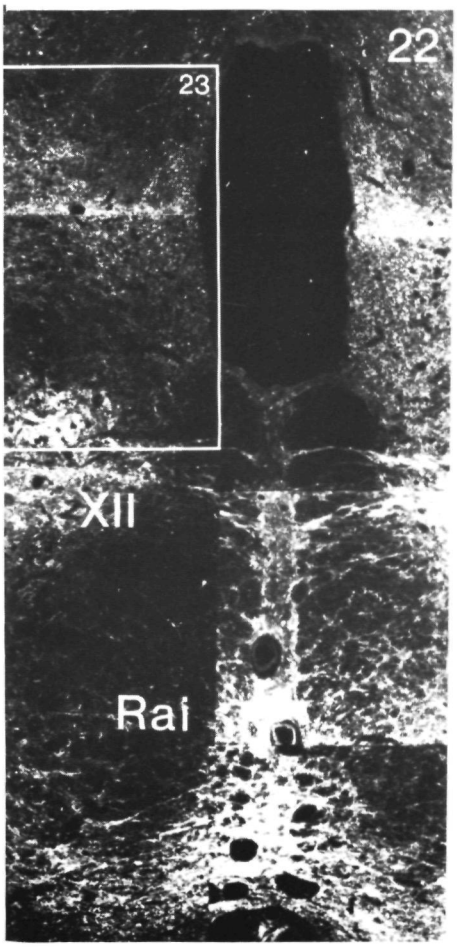
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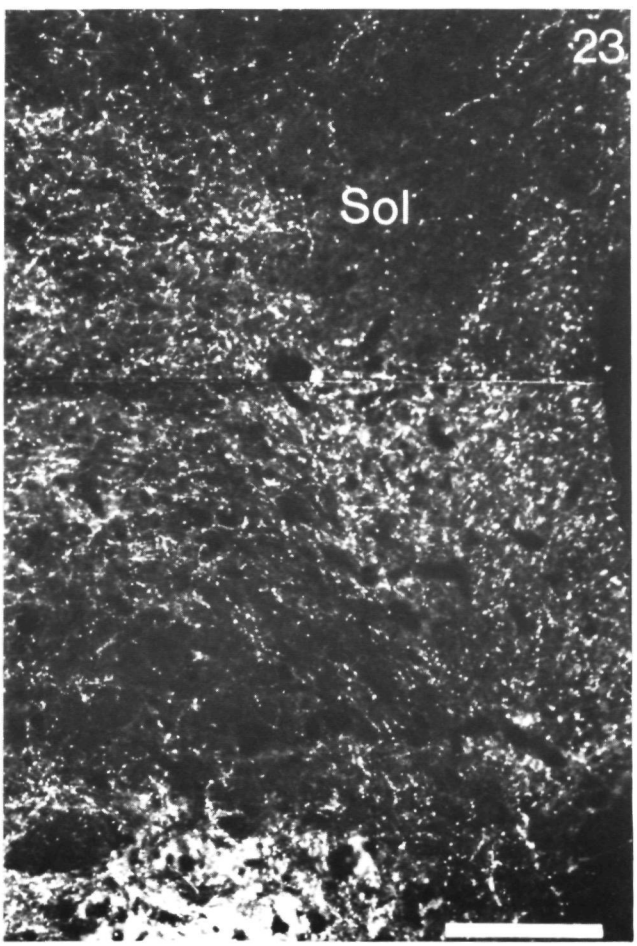
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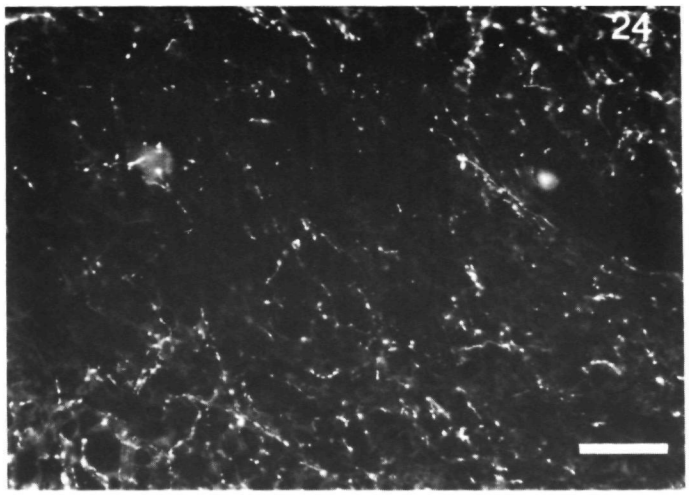
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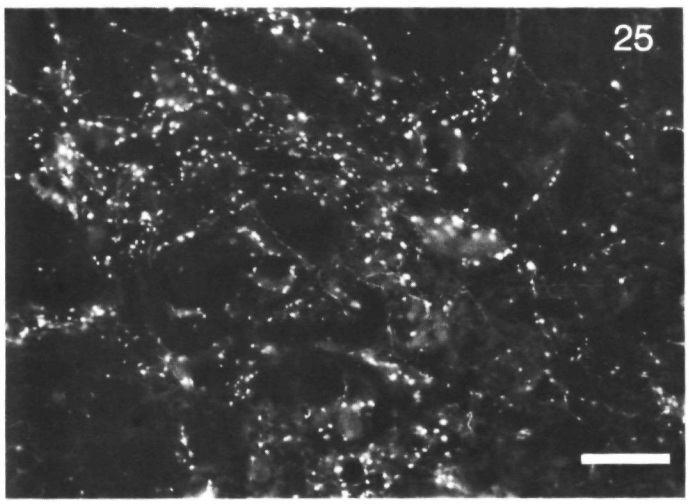
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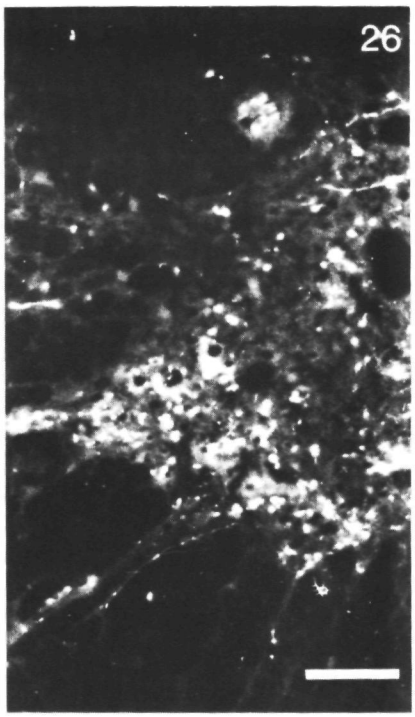
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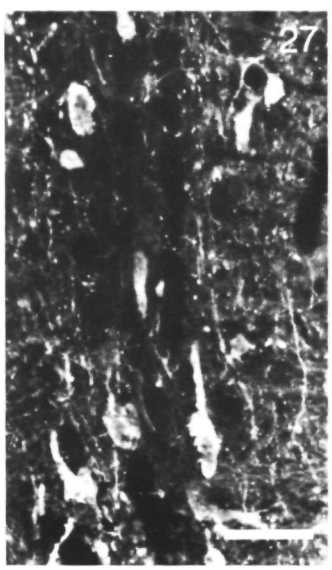
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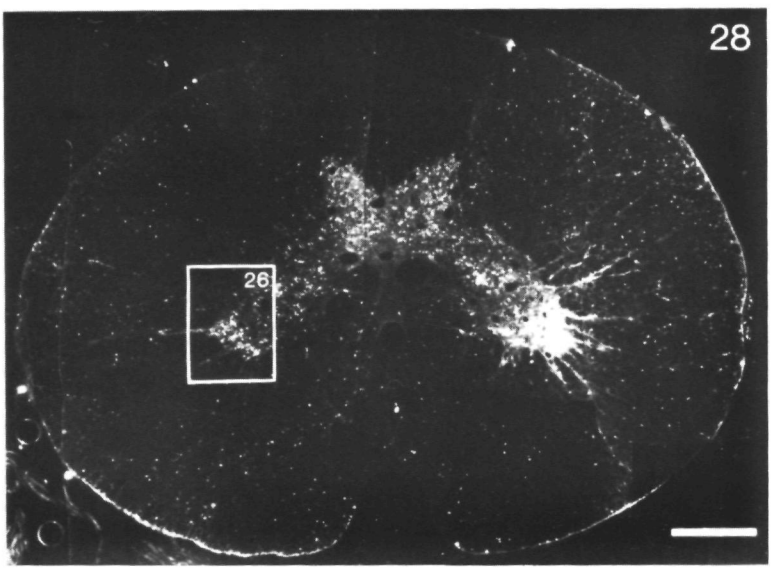
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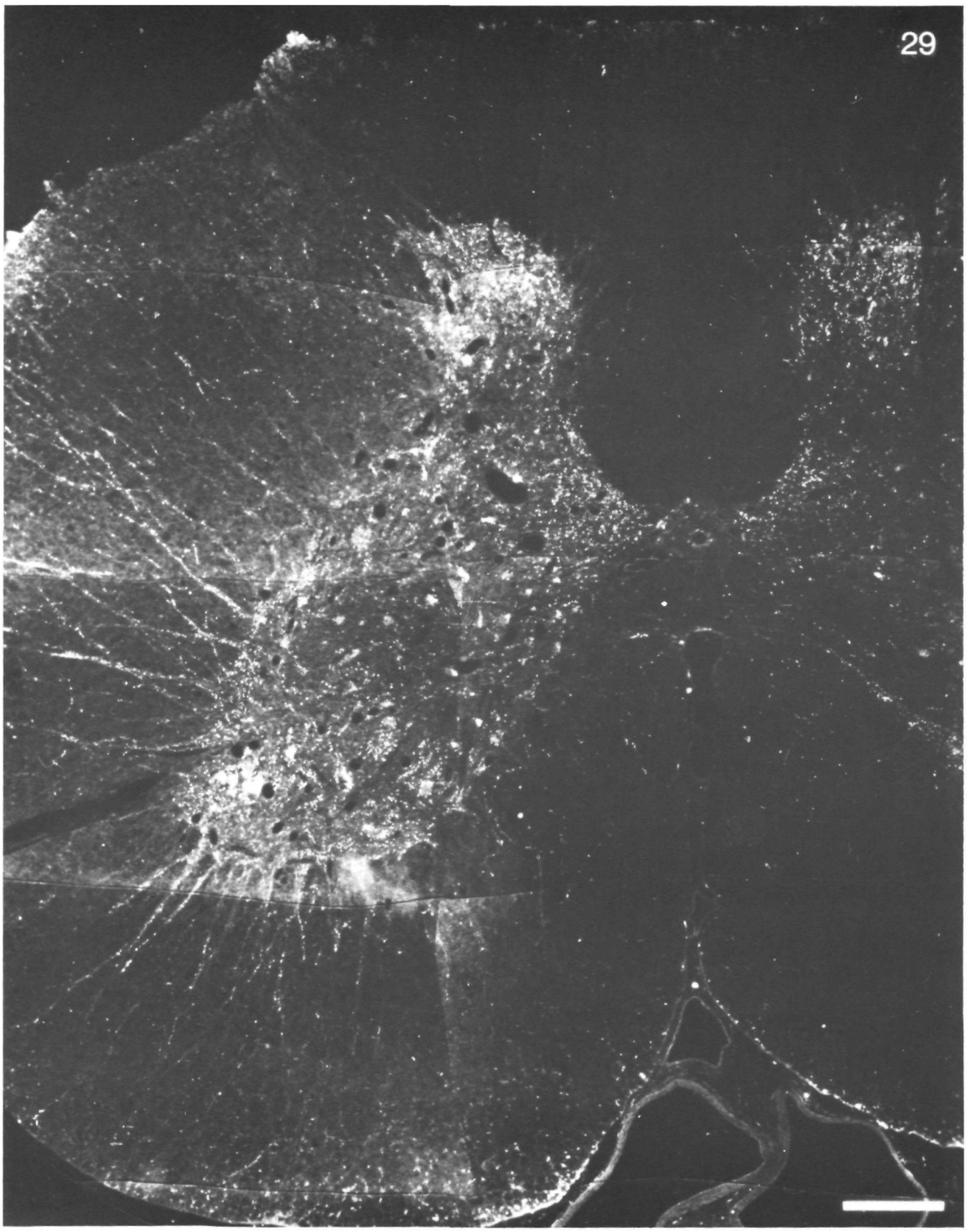

Fig. 29. Cervical intumescence of the spinal cord. General view of the 5-HT immunoreactivity. Varicosities predominantly occur in the dorsal part of the dorsal horn, the intermediate zone and the motoneuron area of the ventral horn. Furthermore, numerous varicosities are related very closely to neuronal processes extending over long distances into the white matter. Relatively few cross-sectioned 5-HT_i fibers are present in the dorsal part of the lateral funiculus and the ventral part of the ventral funiculus. (Bar: 150 μm.)

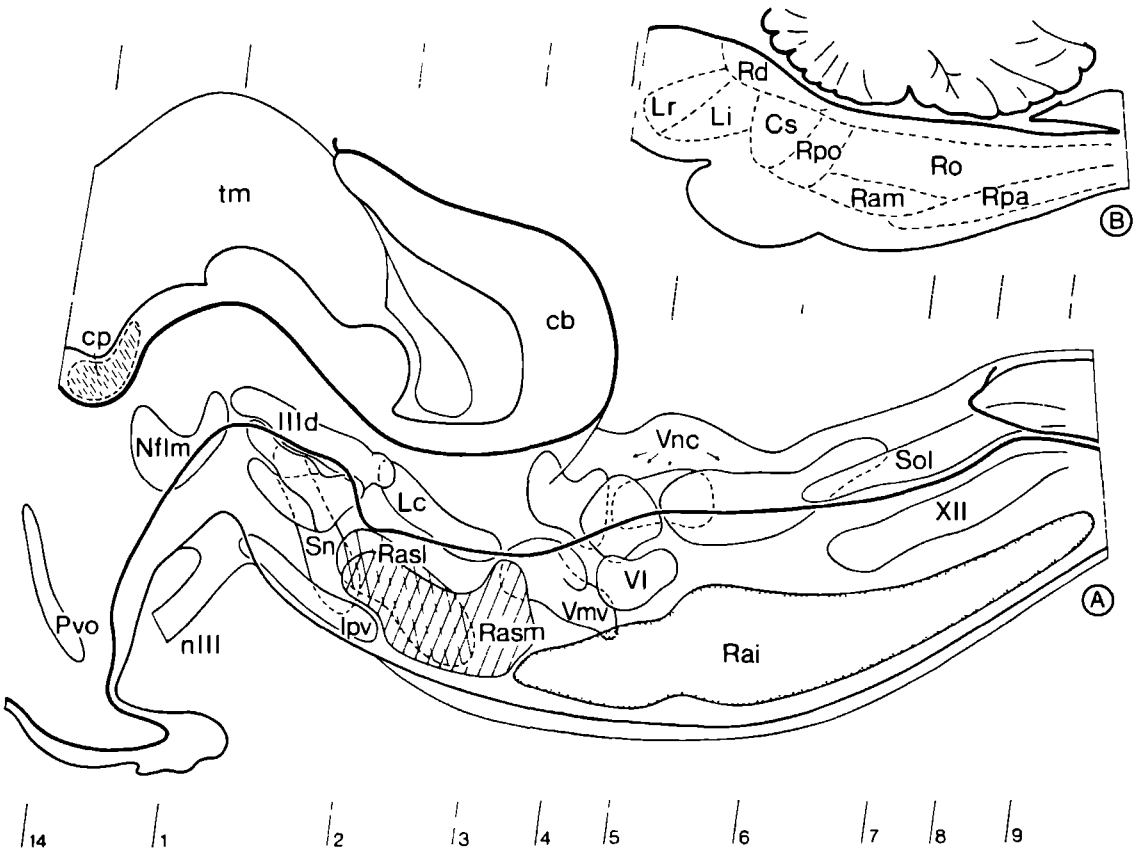

Fig. 30(A). Lateral view of the brain stem of the lizard *Varanus exanthematicus*. Projections of several brain stem cell masses have been indicated in the sagittal plane. The cross-hatched areas indicate the lateral and medial parts of the nucleus reticularis superior respectively, the dotted area represents the nucleus reticularis inferior. Numbers indicate the levels of the sections from which the drawings were made. (B) Lateral view of the cat brain stem, in which the different raphe nuclei of mammals have been indicated (based on Taber Pierce *et al.*[109]).

some extent a cross-reactivity to dopamine [103 105] If rather unspecific pharmacological pretreatments are used to enhance endogenous monoamine levels, together with possibly too low serum dilutions of the 5-HT antibody, dopamine-containing cell bodies are likely to be visualized Since we have used optimal experimental conditions this possible cross-reacting factor can be neglected

Serotonin-immunoreactive nerve fibers and terminals in the brain stem and spinal cord

The projections of the mammalian raphe nuclei have been studied extensively with a variety of techniques Since the retrograde cell degeneration studies of Brodal *et al* [18] it has become clear that mesencephalic raphe nuclei predominantly project rostralwards, medullary groups mainly caudalwards With anterograde degeneration and tracer techniques[10 22 108] it has been observed that ascending raphe projections supply several groups in the mesencephalon (e g the periaqueductal grey), in the diencephalon (hypothalamus, intralaminar and other thalamic nuclei) and in the telencephalon (e g caudate-putamen complex and cerebral cortex) With the formaldehyde-induced fluorescence technique,[76 38 39] immunohistochemistry[61 100] or after intraventricular administration of tritiated serotonin [84] serotonergic fibers have been traced rostrally in a similar way Also in reptiles a rather extensive serotonergic innervation of the mesencephalon (see below), the hypothalamus and telencephalon[46 76 87 117] has been demonstrated Horseradish peroxidase studies in various reptiles (the lizard *Tupinambis nigropunctatus*,[64] the snakes *Thamnophis sirtalis* and *Natrix sipedon*[118]) showed a projection from the nucleus raphes superior to the dorsomedial cortex in the lizard *Varanus exanthematicus* projections from the nucleus raphes superior to the nucleus dorsomedialis thalami and to the habenula[45] were demonstrated In the turtle *Chrysemys picta* Parent[79] showed ascending projections to the basal forebrain arising in laterally situated, presumably 5-HT-containing cells in the caudal midbrain In this respect it is interesting to note that in Kluver–Barrera[51] stained material of the lizards *Tupinambis nigropunctatus* and *Varanus exanthematicus* (H J ten Donkelaar and J G Wolters, unpublished observations) a bundle of coarse fibers can be traced through the ventral part of the mesencephalon (cf Fig 26) and further rostralwards via the medial forebrain bundle to the basal forebrain These fibers seem to arise, at least partly, from the 5-HT, neurons in the ventral and ventromedial mesencephalic tegmentum In the pigeon, Benowitz and Karten[7] showed a distinct, coarse-fiber pathway originating in the nucleus linearis raphe, which courses through the ventral hypothalamus and terminates in a restrictive portion of the contralateral area parahippocampalis

After injections of the fluorescent tracer "Fast Blue" into the rostral mesencephalon[114] extensive ascending projections were found to arise in the nucleus raphes superior including laterally situated cells along the ventral border of the brain stem, but also in the nucleus raphes inferior In the caudal part of the latter nucleus, however, hardly any ascending fibers arise

Extensive serotonergic projections have been demonstrated in the brain stem of *Varanus exanthematicus* In the mesencephalon in particular the 5-HT, innervation of the nucleus profundus mesencephali pars rostralis, the nucleus interpeduncularis and the tectum mesencephali should be mentioned The characteristic 5-HT terminals on dendrites and neurons of the nucleus profundus mesencephali (Fig 15), have been described earlier by Braak *et al* [16] (see their Fig 9) in *Lacerta* species In *Chrysemys picta*[76] and *Clemmys japonica*[117] a dense serotonergic innervation of the central and lateral midbrain tegmentum has been described In mammals no comparable 5-HT terminal area is present, but here, in contrast to what has been found in reptiles, a high density of 5-HT, terminals is present in the substantia nigra [21 84 100] The reptilian counterpart of the substantia nigra, however, is lying considerably more caudally than this densely serotonin-innervated central midbrain tegmental field

The nucleus interpeduncularis receives a dense serotonergic innervation in reptiles (see Refs 76 117, the present study) as in mammals [71 84 94 100] In mammals this projection arises in 5-HT neurons in the nucleus raphes dorsalis [9] The 5-HT, fibers coursing dorsoventrally near the midline at rostral midbrain levels in *Varanus exanthematicus*, as well as the coarse cross-sectioned 5-HT, fibers in the ventral tegmental area (Fig 1) presumably represent the beginning of the reptilian homologue of the transtegmental 5-HT system in mammals [84]

In most vertebrate species (see Refs 82 and 83 for review) the tectum mesencephali appears to be an important target for 5-HT fibers In reptiles most 5-HT terminals are found in the stratum griseum centrale[16 76 117] (the present study) In mammals only the most superficial layer of the colliculus superior receives a distinct serotonergic input [84 100]

The periaqueductal grey at midbrain and isthmic levels receives a dense serotonergic innervation in *Varanus exanthematicus* In mammals this area too receives an input from 5-HT fibers that ascend via the flm [70]

In the various motor nuclei present in the lizard brain stem, a characteristic terminal pattern of 5-HT, fibers is seen a dense plexus of terminals and thin fibers surrounds all motoneurons present in the nuclei of the oculomotor, trochlear, trigeminal, abducens, facial vagal and hypoglossal nerves (Figs 16, 20–23 and 25) A very similar picture is seen in the ventral horn of the spinal cord at all levels the motoneurons are covered with fairly thick serotonergic nerve terminals These observations are supported by similar findings in the spinal cord and certain motor nuclei

in the brain stem of the turtle *Clemmys japonica*,[117] the lizard *Gekko gecko*[46] and in mammals [70 100 121]

At the isthmic level especially cerebellar structures receive a serotonergic input in *Varanus exanthematicus*, e g the cerebellar granular layer (see also Refs 2 and 111) and the cerebellar nuclei Also the region immediately lateral to the locus coeruleus, i e the nucleus parabrachialis, and the dorsolateral border of the brain stem, lateral to the brachium conjuctivum are densely innervated In mammals a moderate number of 5-HT$_1$ terminals is seen in the cerebellar cortex [21 41 70 84 100] The origin of the 5-HT pathways that innervate the mammalian cerebellum is found in all raphe nuclei of the brain stem[96 109] but mainly in the nucleus raphes pontis and raphes obscurus In reptiles a rather modest raphecerebellar projection has been found[3 111] from both nuclei raphes superior and inferior

Numerous regions in the medulla oblongata are seen to receive a serotonergic input in *Varanus exanthematicus*, as in most examined vertebrate species In this lizard most conspicuous labeling of 5-HT$_1$ terminals and fibers is seen in the vestibular nuclear complex (Figs 21 and 24), the nucleus descendens nervi trigemini (Fig 21), the nucleus raphes inferior, the motor nuclei of the cranial nerves, and in the central grey bordering the fourth ventricle, including parts of the nucleus of the solitary tract (Figs 22 and 23) In mammals, apparently, only few 5-HT terminals occur in the vestibular nuclei [38 70 94 100] In contrast, the nucleus descendens nervi trigemini, the periventricular grey, the raphe nuclei and certain areas in the reticular formation are among the most densely innervated of the mammalian brain stem [70 100]

In the spinal cord of *Varanus exanthematicus* the highest density of 5-HT$_1$ terminal structures is found in the dorsal horn, in the intermediate zone, particularly lateral to the central canal, and in the ventral horn in the motoneuron area These observations are in keeping with those in opossum,[28] rat,[8 100] cat[75] and monkey [48 54] However, since in reptiles a lateral horn or intermediolateral cell is missing in the spinal cord, the conspicuous 5-HT innervation of this area as in mammals[8 28 48 53 54 100] is absent in the reptilian spinal cord Kusuma and ten Donkelaar,[56] however, suggested the presence of autonomic preganglionic cell bodies just lateral to the central canal (area X[57]) The dense serotonergic innervation in this area as observed in the spinal segments 15 and 26 (Figs 11 and 12) might therefore include a serotonergic projection to autonomic preganglionic cells It should be noted, however, that a rather similar innervation pattern is seen at cervical and tail levels as well (Figs 10, 13, 28 and 29) In the spinal cord of the stingray *Dasyatis sabina*[92 93] a lateral horn is also missing In this vertebrate, however, only the dorsal and ventral horn receive a serotonergic innervation

In the present study cross-sectioned descending serotonergic fibers to the spinal cord were observed mainly in the dorsolateral and ventral funiculi, in keeping with findings in mammals,[8 26 28 48 53 54 100] and the pigeon [20] The origin of these descending pathways in reptiles has been studied with retrograde tracers Horseradish peroxidase studies[72 112 113 115 123 127] showed rather extensive raphespinal projections arising in the nucleus raphes inferior, particularly in its caudal part, but not in the nucleus raphes superior These data were confirmed after spinal application of the fluorescent tracers "Fast Blue" and "Nuclear Yellow"[114] (J G Wolters, R de Boer-van Huizen and H J ten Donkelaar, in preparation) although more extensive projections from the nucleus raphes inferior were shown than in the horseradish peroxidase experiments no spinal projections were found to arise within the confines of the nucleus raphes superior The most rostrally located raphe cells projecting to the spinal cord were found at the level of the trigeminal motor nucleus After injections of "Fast Blue" into the mesencephalon, and "Nuclear Yellow" into the rostral spinal cord, double-labeled cells, i e neurons with both ascending and descending projections, were observed in the middle part of the nucleus raphes inferior [114] After the application of horseradish peroxidase to the caudal part of the nucleus raphes inferior in the snake *Python regius* (H J ten Donkelaar and G C Bangma, unpublished observations) anterogradely labeled fibers could be traced to the rostral spinal cord via both the lateral and ventral funiculus Terminal structures were observed in the dorsal horn (mainly areas I and II[57]), in the intermediate grey and on motoneurons, in a similar way as described in the present study The funicular trajectory of raphespinal projections in *Varanus exanthematicus* has also been confirmed after the application of horseradish peroxidase slow-release gels to restricted parts of the lateral and ventral funiculus[123] and after injection of [^{3}H]leucine into the nucleus raphes inferior (J G Wolters, J Dederen and H J ten Donkelaar, unpublished observations)

In mammals experimental data obtained from either a combination of the fluorescent retrograde labeling technique and histofluorescence[8 67-69] or a combination of the horseradish peroxidase tracing technique and immunofluorescence[11 24] as well as with anterograde axonal transport of labeled amino acids[4 43 44 67 68] indicate three major components in the descending serotonin system (1) a dorsal pathway, originating in the nucleus raphes magnus and its lateral extension in the nucleus reticularis magnocellularis, projecting ipsilaterally via the dorsolateral funiculus to innervate the dorsal horn, (2) a ventral pathway, arising in the nucleus raphes pallidus and obscurus, and descending ipsilaterally in the ventrolateral and ventral funiculi to innervate the ventral horn and intermediate grey, (3) an intermediate pathway originating in the nucleus raphes obscurus and adjacent reticular formation, descending ipsilaterally in the lateral funiculus to innervate the intermediolateral cell column in the thoracic cord

The above-mentioned data in *Varanus exan-*

thematicus, except for the absence of a distinct projection to an intermediolateral cell column, all fit rather well into this scheme, suggesting a phylogenetic constancy of the serotonergic innervation of the spinal cord

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5

DISTRIBUTION OF SOME PEPTIDES (SUBSTANCE P, [LEU]ENKEPHALIN, [MET]ENKEPHALIN) IN THE BRAIN STEM AND SPINAL CORD OF A LIZARD, VARANUS EXANTHEMATICUS

Abstract — The distribution of substance P and [Leu]- and [Met]enkephalin-immunoreactive cell bodies, fibers and terminal structures in the brain stem and spinal cord of a lizard, *Varanus exanthematicus*, was studied with the indirect immunofluorescence technique, using antibodies to these peptides Substance P-immunoreactive cell bodies were found in the hypothalamus, in a periventricular cell group in the rostral mesencephalon, in the interpeduncular nucleus, in and ventral to the descending nucleus of the trigeminal nerve, in and directly ventral to the nucleus of the solitary tract, scattered in the brain stem reticular formation and in the trigeminal and spinal ganglia A rather widespread distribution of substance P-like immunoreactivity was found in the brain stem and spinal cord, mainly concentrated in striatotegmental projections, in projections related to visceral and/or taste information (nucleus of the solitary tract, parabrachial region), in the descending nucleus of the trigeminal nerve and in the dorsal horn of the spinal cord (areas I and II) In the spinal cord also around the central canal (area X and adjacent parts of area V-VI) a distinct substance P innervation was found The ventral horn receives only a very sparse substance P innervation

The distribution of [Leu] and [Met]enkephalin in the brain stem and spinal cord of *Varanus exanthematicus* is less impressive than that of substance P Enkephalinergic cell bodies were found particularly in the caudal hypothalamus Less extensive populations of enkephalinergic cell bodies were found in the vestibular nuclear complex, in the nucleus of the solitary tract, in and around the descending nucleus of the trigeminal nerve and throughout the rhombencephalic reticular formation Enkephalins are likely to be present in efferent projections of the striatum, in projections related to taste or visceral information (nucleus of the solitary tract, parabrachial region) and in descending pathways to the spinal cord Enkephalinergic fibers are present in the lateral funiculus and enkephalin-immunoreactive cell bodies are found in the reticular formation, particularly the inferior reticular nucleus which is known to project to the spinal cord In the spinal cord enkephalinergic terminal structures were found especially in the superficial layer of the dorsal horn (areas I and II) and around the central canal The ventral horn including the motoneuron area receives only a relatively sparse enkephalinergic innervation

In many respects the distribution of substance P- and [Leu]- and [Met]enkephalin-immunoreactivity in the brain stem and spinal cord of the lizard *Varanus exanthematicus* is comparable to that in mammals

Introduction

Peptides such as substance P and the endogenous opioid peptides [Leu]- and [Met]enkephalin are considered to play key roles as transmitters or modulators in the central and peripheral nervous system Since von Euler and Gaddum's (1931) paper more than half a century of research on substance P (see Porter and O'Connor 1982; Pernow 1983) has brought forward a detailed knowledge of the distribution and properties of this peptide. The distribution of substance P neurons, fibers and terminal structures has been studied in various vertebrates, particularly in the rat (Nilsson et al 1974, Hokfelt et al 1975b; 1977b, Cuello and Kanazawa 1978, Ljungdahl et al. 1978a, b; Cuello et al. 1982; Inagaki et al. 1982; Sakanaka et al 1982), but also in amphibians (the frog *Rana catesbeiana* Inagaki et al 1981, the newt *Triturus cristatus*· Taban and Cathieni 1983) Throughout terrestrial vertebrates high concentrations of substance P were found e g. in the striatonigral projection (see also· Brauth et al. 1983; Reiner et al 1983, 1984) and in the dorsal horn of the spinal cord (see Hunt 1983 for review).

Endogenous opioid peptides like [Leu]- and

[Met]enkephalin have been implicated in a number of functions including autonomic and endocrine regulations, and nociception (see Hughes 1983, Akil et al 1984) A detailed account of the central distribution of enkephalin-immunoreactive cell bodies, fibers and terminal structures has been provided for the rat (Elde et al 1976, Hokfelt et al 1977a, Simantov et al 1977, Watson et al 1977, Sar et al 1978, Uhl et al 1979, Walmsley et al 1980, Finley et al 1981, Khachaturian et al 1983, Williams and Dockray 1983) and for monkey (Haber and Elde 1982)

Although in reptiles a number of studies on the central nervous distribution of peptides have been carried out (Naik et al 1981, Brauth et al 1983, Brauth 1984, Reiner et al 1984), these studies mainly focussed on the telencephalon and the substance P-containing striatonigral pathway So far no comprehensive survey of the distribution of substance P-containing or enkephalin-immunoreactive cell bodies, fibers and terminal structures is available for the lizard brain stem and spinal cord In the present study the distribution of substance P and [Leu]- and [Met]enkephalin is described for the brain stem and spinal cord of the lizard *Varanus exanthematicus* In previous studies (Wolters et al 1984, 1985) the brain stem and spinal distribution of catecholaminergic and serotonergic cell bodies, fibers and terminal structures has been described

Materials and techniques

Experimental animals

Six lizards (*Varanus exanthematicus*) were used, varying in weight from 300 to 550 g, with a total length of 52-70 cm and a snout-vent length of 26-34 cm The animals were housed in an air-conditioned room with a fixed temperature and dark-light cycle (10 h, 18 °C / 14 h, 24 °C) Four animals received colchicine (2 mg dissolved in 1 ml 0 9% sodium chloride containing 2% ascorbinic acid) 48 h before being sacrificed (0 8 mg/40 ul in the cisterna magna and 0 4 mg/200 ul intraperitoneally) Additional doses of colchicine were administered 24 h (0 8 mg/400 μl i p) and 2 h (1 2 mg/600 μl i p) before the animals were perfused

Preparation of tissues

The animals were anaesthetized with a mixture of oxygen, nitrous oxide, and Ethrane, followed by an intravenous injection of 0 4 ml Nembutal Then they were perfused through the heart with ice-cold (4 °C) calcium-free Tyrode's solution (Ca^{2+}) for 2 min , immediately followed by 500 ml ice-cold 4% paraformaldehyde dissolved in 0 1 M sodium phosphate buffer, pH 7 3, and post-fixed in the same fixative for 18-24 h The brain stem, from the caudal part of the diencephalon up to the spinal cord, and pieces taken at four levels of the spinal cord (viz , the 6th, 15th, 26th, and 38th spinal segments, representing respectively cervical, thoracic, lumbar and tail levels) were frozen with powdered carbon dioxide gas Transversal sections were cut at 10 μm on a cryostat (Dittes, Heidelberg, F R G) Series of sections collected at twenty-two levels through the brain stem and the four levels of the spinal cord were mounted on glass slides coated with a chromalum-gelatin solution (0 05 g chromalum and 0 5 g gelatin in 100 ml distilled water) and stored at -70 °C pending staining In the brain stem the interval between the first sections of two consecutive series was about 500 μm

Immunofluorescence procedure

Antisera, kindly provided by Dr P C Emson, were raised in rabbits using substance P, [Leu]- or [Met]enkephalin linked to bovine serum albumin with carbodiimide Radio-immunoassay showed that the anti-substance P serum was C-terminal directed Cross-reactivity of the antiserum to [Met]enkephalin with [Leu]enkephalin was about 1%, whereas the antiserum to [Leu]enkephalin showed a cross-reaction to [Met]enkephalin of about 10% (personal communication of Dr P C Emson). Before use all serum samples were treated with bovine serum albumin (4 mg/ml)

Four series of sections were stained according to the indirect immunofluorescence procedure described by Coons (1958) The sections were first rinsed in phosphate buffered saline (PBS) at room temperature for 30 min Then, series of sections were incubated with the antiserum to substance P, [Leu]- or [Met]enkephalin or by serum from non-immunized rabbits for 18 h at 4 °C All sera were diluted 1 400 with PBS containing 0.1% Triton X-100 After rinsing in PBS for 30 min at room temperature, the sections were incubated with fluorescein isothyocyanate-labeled sheep anti-rabbit immunoglobulins (Statens Bakteriologiska Laboratorium, Stockholm, Sweden) diluted 1 16 with PBS, also containing 0 1% Triton X-100 (30 min, room temperature) Finally, they were rinsed in PBS for 30 min at room temperature, mounted in glycerin-PBS (3.1) and stored at -20 °C

Evaluation and presentation of results

The sections were examined with a Zeiss Universal microscope, equipped with incident illumination for fluorescence Kodak Tri-X film was used for photomicrography, having exposure times between 15 and 40 s The distribution of immunoreactive cell bodies, fibers and terminals is presented in schematic drawings of cryostat sections stained with

cresylechtviolet The density of fluorescent fibers and terminals was classified subjectively into five categories **(a)** no or hardly any fluorescence, **(b)** low density; **(c)** medium density; **(d)** high density; **(e)** very high density

Results

The distribution of substance P-immunoreactive and [Leu]- and [Met]enkephalin-immunoreactive cell bodies, nerve fibers and terminals in the brain stem and spinal cord of the lizard *Varanus exanthematicus* will be described from rostral to caudal, starting at the level of the posterior commissure, down to the caudalmost part of the medulla oblongata at the level of the obex, followed by four selected segments of the spinal cord The terminology used is mainly based on Butler and Northcutt (1973), Cruce and Nieuwenhuys (1974), ten Donkelaar and Nieuwenhuys (1979) and Newman and Cruce (1982)

The results are markedly different between colchicine-treated and non-treated lizards Substance P- and enkephalin-immunoreactive cell bodies were only observed after colchicine treatment

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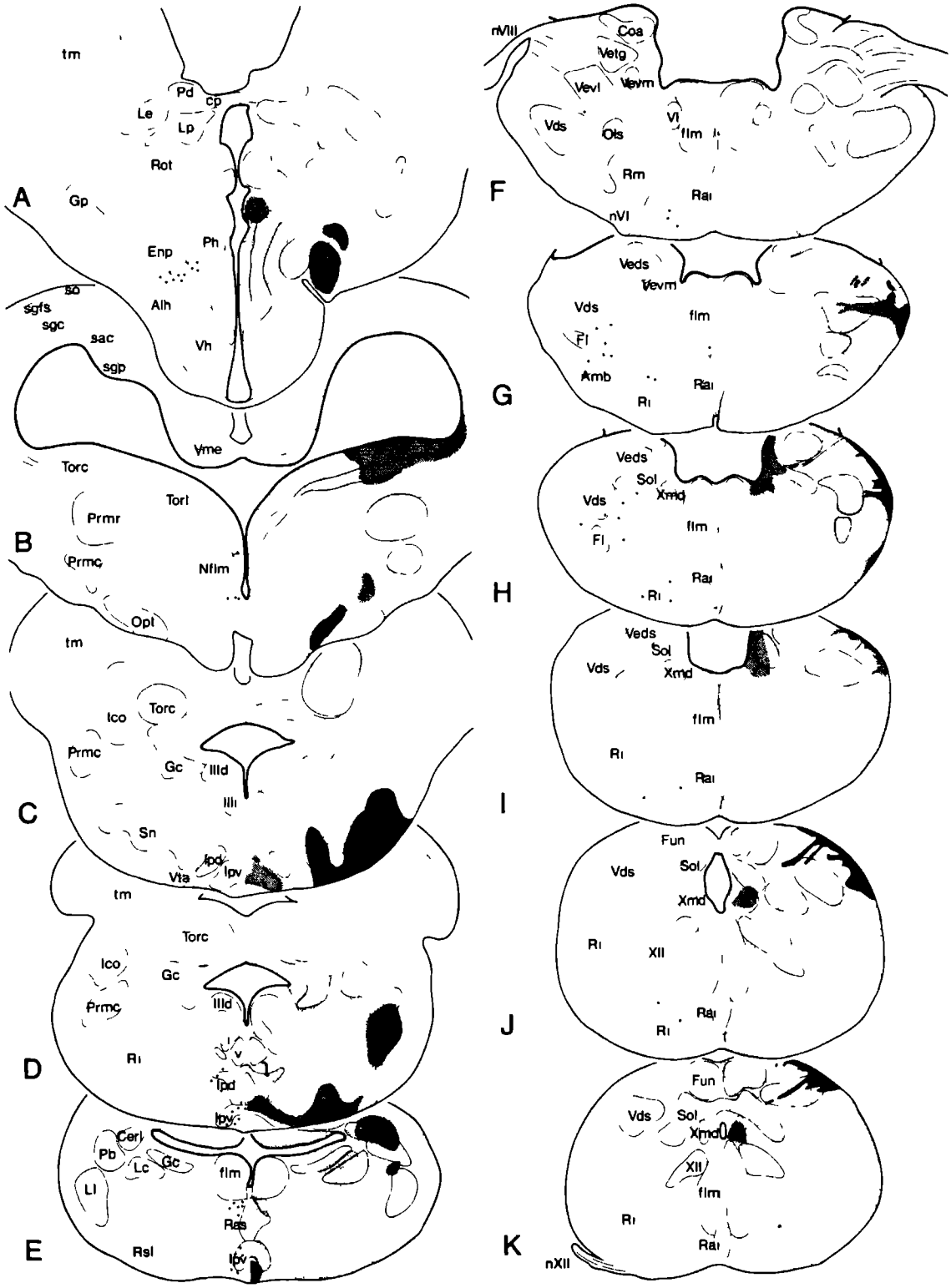
Abbreviations used in the figures

Alh	area lateralis hypothalami
Amb	nucleus ambiguus
cereb	cerebellum
Cerl	nucleus cerebellaris lateralis
Cerm	nucleus cerebellaris medialis
Coa	nucleus cochlearis angularis
cc	central canal
cp	commissura posterior
dh	dorsal horn
Enp	nucleus entopeduncularis posterior
fd	funiculus dorsalis
Fl	nucleus funiculi lateralis
flm	fasciculus longitudinalis medialis
Fun	nucleus funiculi dorsalis
Gc	griseum centrale
ggl	V ganglion nervi trigemini
ggl	VII ganglion nervi facialis
gl	lamina granularis cerebelli
Gp	nucleus geniculatus pretectalis
Ico	nucleus intercollicularis
IIId	nucleus nervi oculomotorii, pars dorsalis
IIIi	nucleus nervi oculomotorii, pars intermedia
IIIv	nucleus nervi oculomotorii, pars ventralis
Ipd	nucleus interpeduncularis, pars dorsalis
Ipv	nucleus interpeduncularis, pars ventralis
Lc	locus coeruleus
Le	nucleus lentiformis thalami, pars extensa
L-ENK	[Leu]enkephalin
lfb	lateral forebrain bundle
Ll	nucleus lemnisci lateralis
Lp	nucleus lentiformis thalami, pars plicata
M-ENK	[Met]enkephalin
Nflm	nucleus of the flm
nII	nervus opticus
nIII	nervus oculomotorius
nV	nervus trigeminus
nVI	nervus abducens
nVII	nervus facialis
nVIII	nervus vestibulocochlearis
nXII	nervus hypoglossus
Ols	oliva superior
Opt	nucleus opticus tegmenti (= nucleus of the basal optic root)
Pb	parabrachial region
Pd	nucleus posterodorsalis
Ph	nucleus periventricularis hypothalami
Prmc	nucleus profundus mesencephali, pars caudalis
Prmr	nucleus profundus mesencephali, pars rostralis
Rai	nucleus raphes inferior
Ras	nucleus raphes superior
Ri	nucleus reticularis inferior
Ris	nucleus reticularis isthmi
Rm	nucleus reticularis medius
Rot	nucleus rotundus
Rs	nucleus reticularis superior
Rsl	nucleus reticularis superior, pars lateralis
sac	stratum album centrale
sgc	stratum griseum centrale
sgfs	stratum griseum et fibrosum superficiale
sgp	stratum griseum periventriculare
Sn	substantia nigra
so	stratum opticum
Sol	nucleus tractus solitarii
SP	substance P
sp ggl	spinal ganglion
Str	corpus striatum
tm	tectum mesencephali
Torc	nucleus centralis, torus semicircularis
Torl	nucleus laminaris, torus semicircularis
Vds	nucleus descendens nervi trigemini
Vedl	nucleus vestibularis dorsolateralis
Veds	nucleus vestibularis descendens
Vetg	nucleus vestibularis tangentialis
Vevl	nucleus vestibularis ventrolateralis
Vevm	nucleus vestibularis ventromedialis
Vh	nucleus ventralis hypothalami
VI	nucleus nervi abducentis
vIII	third ventricle
vIV	fourth ventricle
Vmd	nucleus motorius nervi trigemini, pars dorsalis
Vmv	nucleus motorius nervi trigemini, pars ventralis
Vme	nucleus mesencephalicus nervi trigemini
Vpr	nucleus princeps nervi trigemini
Vta	ventral tegmental area
XII	nucleus nervi hypoglossi
Xmd	nucleus motorius dorsalis nervi vagi
5-HT	serotonin (5 hydroxy tryptamine)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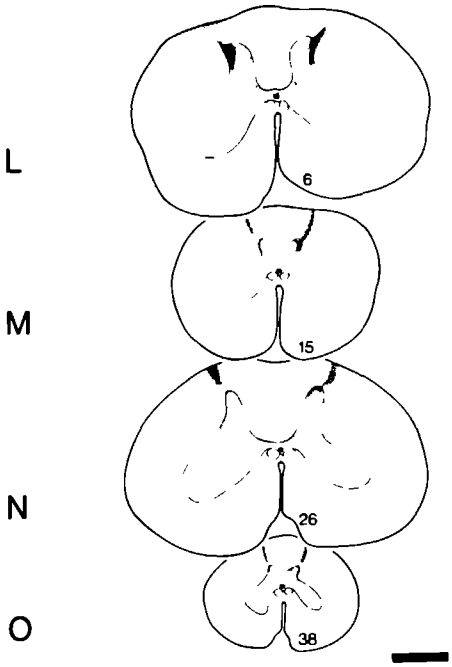


Distribution of substance P-immunoreactive cell bodies, fibers and terminals in the brain stem and spinal cord

In the caudal part of the *diencephalon* (Fig. 1 A) substance P-immunoreactive (SP_I) cell bodies are found in the dorsal part of the area lateralis hypothalami in and ventral to the posterior entopeduncular nucleus, and in the nucleus periventricularis hypothalami. The latter cell group is continuous with the periventricular SP_I cell group in the rostral mesencephalon (Figs. 1 B, 5). In the lateral hypothalamus SP_I fibers are found in a very high density in the ventral peduncle of the lateral forebrain bundle (Figs. 1 A, 4). Several fibers take a diagonal course more or less perpendicular to the brain stem surface, without reaching it. Ventromedial to the lateral forebrain bundle a moderate number of fine varicosities is found. In the medial hypothalamus very fine SP_I varicosities are present throughout the nucleus periventricularis hypothalami. Particularly in the dorsal half of this nucleus a high density of terminal structures as well as thin and very thin varicose fibers are found.

In the pineal organ bundles of numerous thin varicose and non-varicose fibers form a dense SP_I plexus. No cell bodies are found (Fig. 2). SP_I fibers can be traced into the habenula as well as into the leptomeninx (Fig. 3).

In the *mesencephalon* SP_I cell bodies are present at two sites: 1) as a large cluster of medium-sized perikarya in the periventricular grey of the rostral mesencephalon (Figs. 1 B, 5), described as nucleus of the fasciculus longitudinalis medialis (Tuge 1932); 2) in the caudal mesencephalic tegmentum where two smaller groups of SP_I neurons are found: a) throughout the nucleus interpeduncularis pars ventralis (Figs. 1 C-E, 6), b) in the dorsolateral part of the nucleus interpeduncularis pars dorsalis (Fig. 1 D) continuous with SP_I neurons in and around the nucleus raphes superior (Fig. 1 E). In these mesencephalic SP_I cell groups only sparse neuronal processes (Fig. 6) are seen in transverse sections.

The highest density of SP_I fibers in the mesencephalic tegmentum is found in the direct vicinity of the substantia nigra (Figs. 1 C, D, 10), particularly ventrolateral to the cluster of catecholaminergic cell bodies (see Wolters et al. 1984 Figs. 3, 14). More medially, in the area between the ventromedial pole of the substantia nigra and the oculomotor nerve fibers, a rather extensive SP_I innervation is also present. The SP_I fiber bundle lateral to the substantia nigra is continuous with the ventral peduncle of the lateral forebrain bundle as noted in the caudal part of the diencephalon (Figs. 1 A, 4). Its SP_I component can be traced through the mesencephalon (Fig. 1 B-D) and splits up into a dorsal bundle (Fig. 1 D) coursing in the direction of the parabrachial region (Figs. 1 E, 8), and a ventral part which fades out in the ventral isthmic tegmentum.

A high density of very fine SP_I varicosities is found in the periventricular grey, especially ventral to the lateral recess of the third ventricle (Fig. 1 B). This area also contains thin varicose fibers coursing in many directions. More medially, the periventricular zone is less densely innervated. The mesencephalic periventricular SP_I plexus is directly continuous with the one bordering the third ventricle in the caudal diencephalon (Figs. 1 A, 4). Low to moderate densities of SP_I varicosities are seen in several parts of the mesencephalic tegmentum, e.g. the griseum centrale (Fig. 1 C, D), the dorsal part of the nucleus centralis of the torus semicircularis (Fig. 1 B), the nucleus profundus mesencephali pars rostralis and pars caudalis (Fig. 1 B) and the nucleus interpeduncularis (Figs. 1 C-E, 6), in particular the caudal portion of its pars ventralis (Figs. 1 E, 6). A medium density of fine varicosities is found in a small area of the griseum centrale near the lateral corner of the aqueductus cerebri (Fig. 1 D), which contains both catecholaminergic (see Wolters et al. 1984 Fig. 4) and [Leu]- and [Met]enkephalin-

Fig. 1 A-O. Schematic representation of substance P immunoreactivity as observed in representative transversal sections of the brain stem and spinal cord of colchicine-treated lizards (*Varanus exanthematicus*). At the right, the density of SP_I fibers and varicosities is indicated, subjectively classified into five categories: *1.* no or hardly any fluorescence; *2.* low density; *3.* medium density; *4.* high density; *5.* very high density. At the left dots indicate SP_I cell bodies in each section. Numbers within the confines of Fig. 1 L-O indicate spinal levels of the pictured sections. (Bar: 0,5 mm)

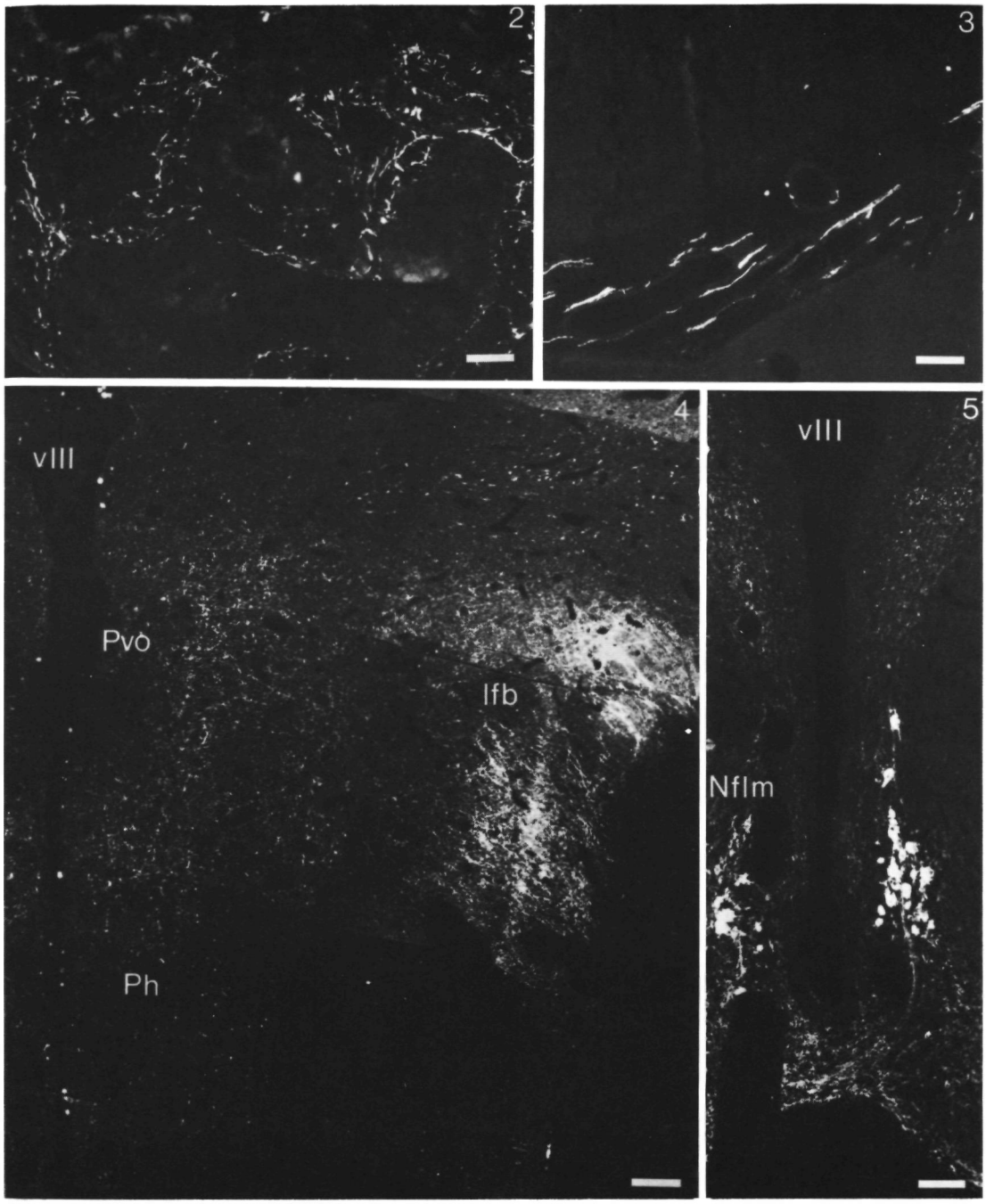


Fig. 2. Diencephalon. Dense SP_i fiber plexus in the pineal organ. (Bar: 30 μm) **Fig. 3.** Bundles of thin varicose and non-varicose SP_i fibers, as found throughout the lepto meninx, here coursing between the tectum mesencephali and the telencephalic hemisphere. (Bar: 30 μm) **Fig. 4.** Diencephalon. General view of SP immunoreactivity in the hypothalamus of a non-treated lizard. A very high density of SP_i fibers is seen in the lateral forebrain bundle (lfb). Medially, especially in the dorsal part of the nucleus periventricularis hypothalami, numerous terminal structures and varicose fibers are found. The SP_i cellular elements seen in the ependymal layer, are not neuronal, but most probably represent histiocytes or mast-cells. (Bar: 90 μm) **Fig. 5.** Rostral mesencephalon. SP_i neurons in the nucleus of the flm. (Bar: 90 μm)

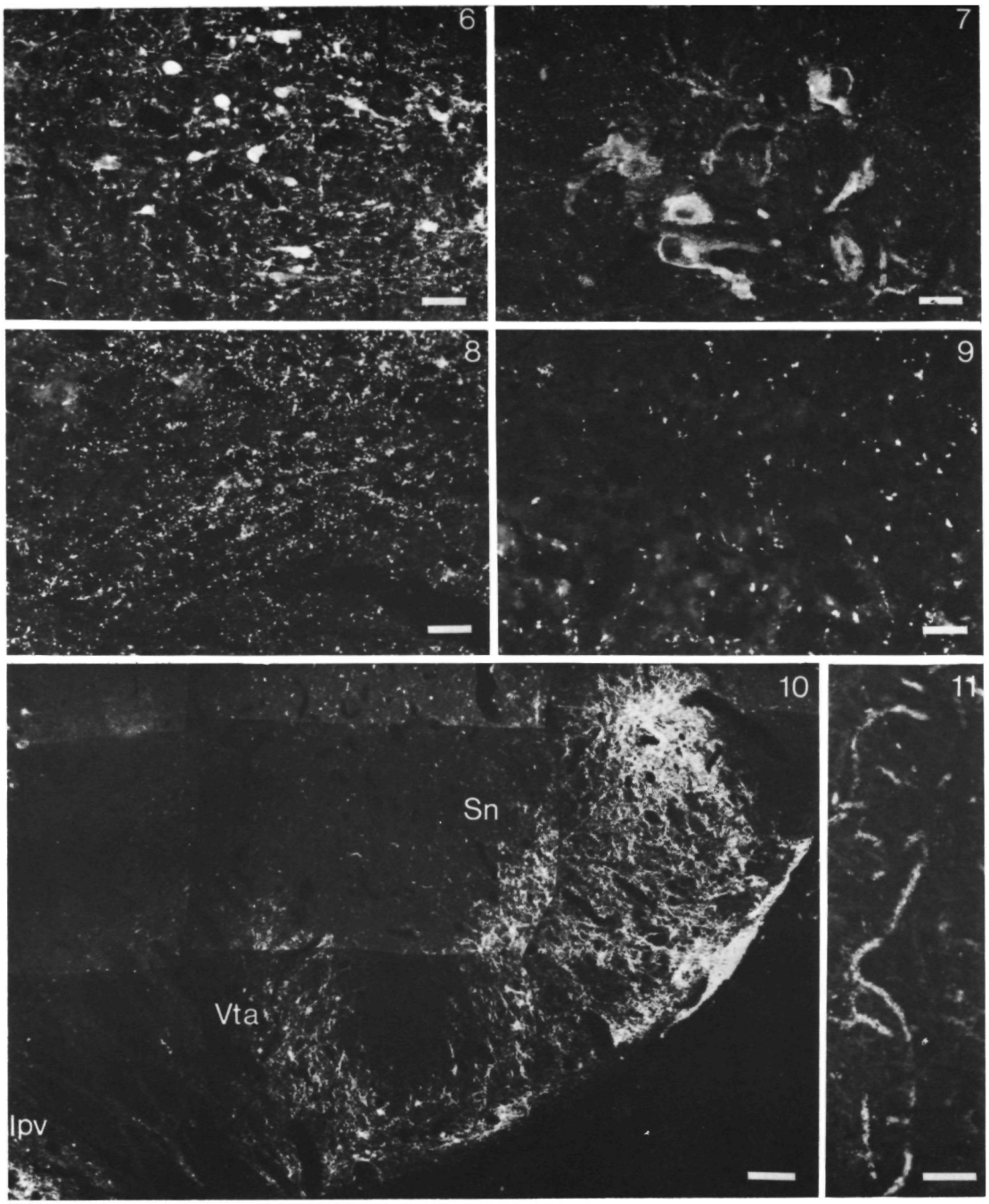


Fig. 6. Caudal mesencephalon. Small SP_i neurons and numerous fine varicose structures in the nucleus interpeduncularis, pars ventralis. (Bar: 20 μm) **Fig. 7.** Rostral rhombencephalon. SP_i motoneurons in the nucleus motorius nervi trigemini, as seen in only one of the colchicine-treated lizards. (Bar: 20 μm) **Fig. 8.** Isthmic tegmentum. Dense plexus of fine and some medium-sized SP_i varicosities just medial to the brachium conjunctivum (bc). (Bar: 20 μm) **Fig. 9.** Cerebellum. Medium density of medium-sized to thick SP_i varicosities in the granular layer. (Bar: 20 μm) **Fig. 10.** Caudal mesencephalon. Very high density SP_i fiber plexus and terminal structures, covering the ventral part of the substantia nigra and ventral tegmental area (Vta); particularly in the latter the form of medium-sized perikarya, covered with SP_i structures, can easily be recognised. In the lower left corner some of the SP_i neurons in the ventral part of the interpeduncular nucleus are visible. In the upper left corner autofluorescent neurons of the nucleus oculomotorius are seen. (Bar: 90 μm) **Fig. 11.** Tectum mesencephali. Thick bulbous SP_i fibers form a tortuous plexus of medium density in the superficial (pictured) and deepest part of the stratum griseum et fibrosum superficiale. (Bar: 20 μm)

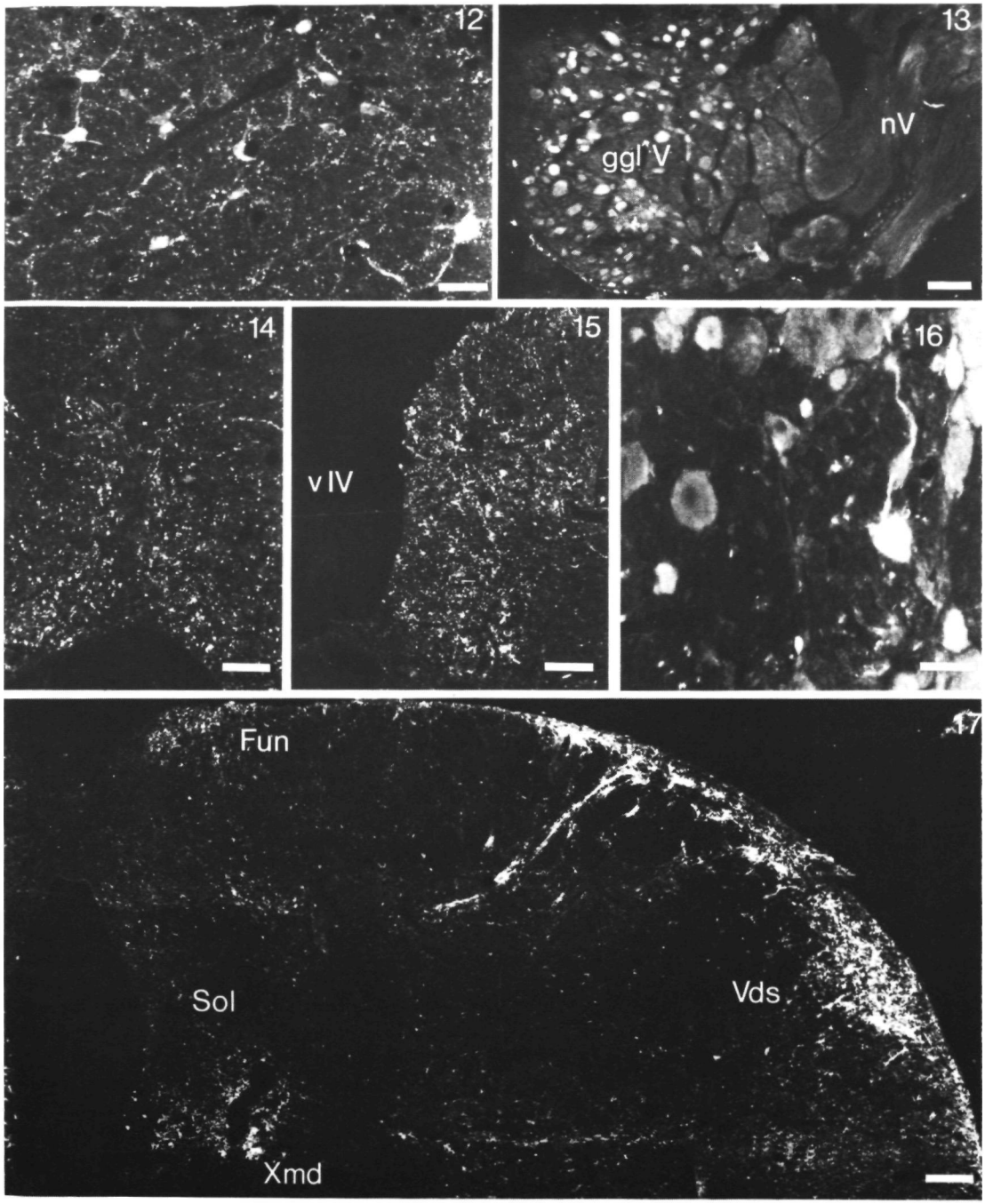


Fig. 12. Rostral rhombencephalon. SP_i neurons ventral to the nucleus descendens nervi trigemini and a medium to high density innervation by fine SP_i varicosities. (Bar: 40 μm) **Fig. 13.** Trigeminal ganglion. Several SP_i neurons of varying fluorescence intensity. (Bar: 30 μm) **Fig. 14.** Caudal rhombencephalon. Very fine to medium-sized varicosities innervating the ventral part of the nucleus raphes inferior. (Bar: 25 μm) **Fig. 15.** Caudal rhombencephalon. Fine to fairly thick varicosities innervating the nucleus tractus solitarii. (Bar: 90 μm) **Fig. 16.** Spinal ganglion (detail). Some SP_i ganglion cells of varying fluorescence intensity and some very fine and medium-sized varicosities; a fairly thick, non-varicose SP_i fiber is seen, originating from a highly fluorescent SP_i neuron. (Bar: 20 μm) **Fig. 17.** Caudal rhombencephalon. General view of the SP immunoreactivity in the dorsal part of the caudal brain stem. Note the very high density SP_i fiber plexus at the dorsolateral border, and the SP_i fiber bundle coursing in the direction of the densely innervated nucleus tractus solitarii. (Bar: 90 μm)

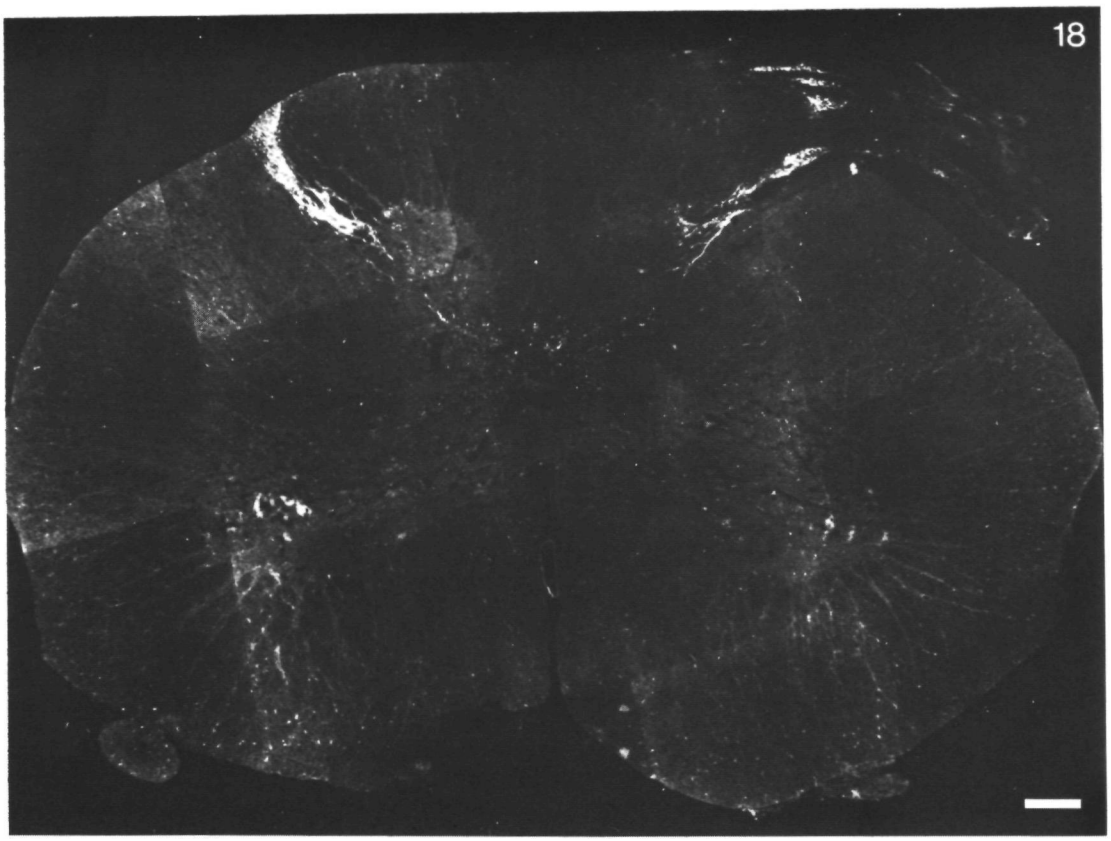


Fig. 18. Lumbar intumescence of the spinal cord. General view of the SP immunoreactivity. Note the very high density SP_i fiber plexus entering via the dorsal roots, innervating the dorsalmost and lateral parts of the dorsal horn (areas I/II), as well as area X, dorsolateral to the central canal. (Bar: 135 μm)

immunoreactive neurons (Fig. 20 C).

Several layers of the tectum mesencephali (Figs. 1 B-D, 11) receive a SP_i innervation. Two layers of fairly thick bulbous SP_i fibers, which for a considerable part course in the transversal plane, form a tortuous plexus of medium density in the most superficial and in the deepest part of the stratum griseum et fibrosum superficiale. Low density thin SP_i fibers are present in between these two layers. A low density of medium-sized varicosities is present in the stratum griseum periventriculare surrounding the lateral recess of the third ventricle. In the stratum album centrale a few SP_i varicosities occur.

At the *isthmic* level (Fig. 1 E) a high density of fine and some medium-sized varicosities is found in the dorsolateral part of the isthmic tegmentum, just medial to the brachium conjunctivum, extending into the parabrachial region and the nucleus cerebellaris lateralis (Fig. 8). In the cerebellum a medium density of mainly medium-sized to thick varicosities is seen in the granular layer (Fig. 9). In and just dorsal to the locus coeruleus (Fig. 1 E) a medium density of brightly fluorescent fibers and terminals is observed. Low amounts of fine varicosities occur in the central part of the nucleus reticularis superior and in the ventralmost part of the isthmic tegmentum.

In the *rhombencephalon* SP_i cell bodies, fibers and terminal structures are found particularly in its caudal part, the medulla oblongata (Fig. 1 G-K). SP_i cell bodies are present just ventral to the ventrolateral vestibular nucleus (Fig. 1 F), in and ventral to the nucleus descendens nervi trigemini (Figs. 1 F-H, 12), in and directly ventral to the nucleus of the solitary tract (Fig. 1 H) and scattered throughout the nucleus reticularis medius (Fig. 1 F) and nucleus reticularis inferior (Fig. 1 G-J). Rarely a SP_i neuron was found in the nucleus raphes inferior (Fig. 1 G). Furthermore, SP_i neurons were observed in the trigeminal ganglion (Fig. 13). In one of the four colchicine-treated animals large SP_i perikarya were found in the motor nucleus of the trigeminal nerve (Fig. 7).

A very high density plexus of SP_i varicosities and fibers is present at the dorsolateral border of the brain stem throughout the medulla oblongata (Fig. 1 G-K). From this plexus of fine and medium-sized varicosities and thin to fairly thick varicose and non-varicose fibers, many fibers arise, which course in a medioventral direction until they reach the nucleus

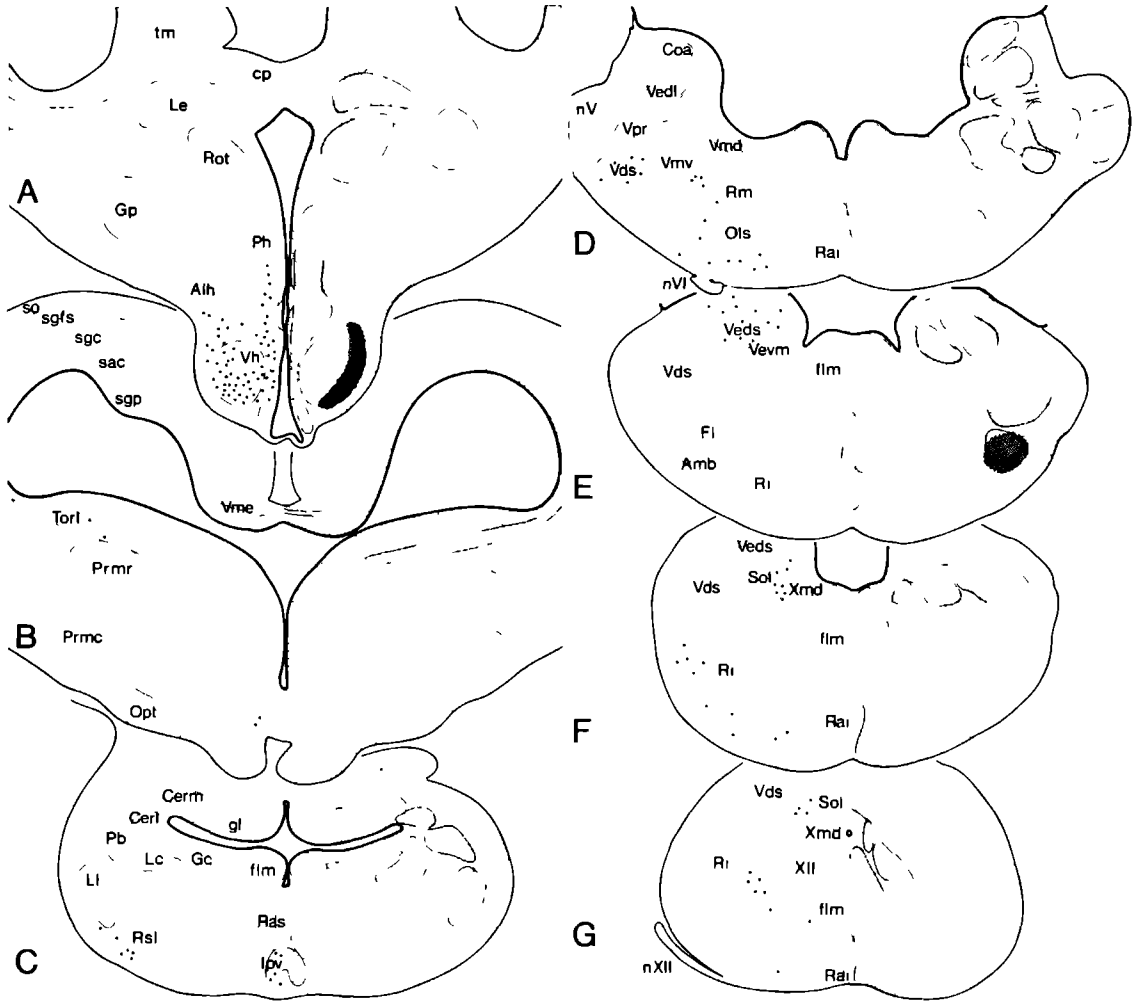
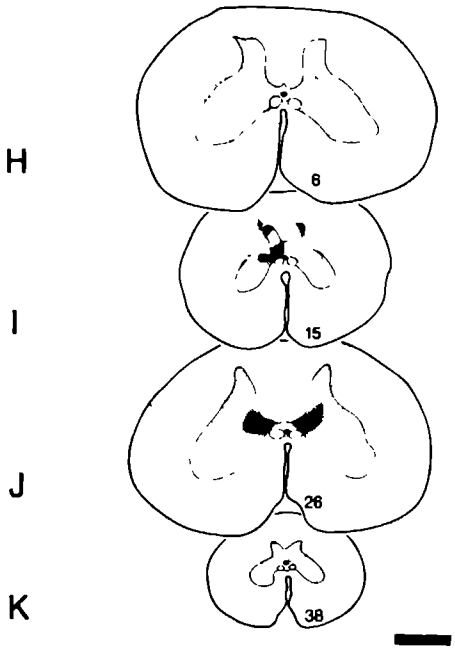


Fig. 19 A-K. Schematic representation of L-ENK immunoreactivity as observed in representative transversal sections of the brain stem and spinal cord of colchicine-treated lizards (*Varanus exanthematicus*). At the right, the density of L-ENK$_i$ fibers and varicosities is indicated, subjectively classified into five categories: *1.* no or hardly any fluorescence; *2.* low density; *3.* medium density; *4.* high density; *5.* very high density. At the left dots indicate L-ENK-immunofluorescent cell bodies in each section. Numbers within the confines of Fig. 19 H-K indicate spinal levels of the pictured sections. (Bar: 0,5 mm)

descendens nervi trigemini (Figs. 1 G-K, 17). More caudally in the medulla oblongata some of these fibers can be traced to the nucleus of the solitary tract (Figs. 1 H, J, K, 17).

A high density of fine to fairly thick SP$_i$ varicosities is present in the periventricular grey, including large parts of the nucleus of the solitary tract and the nucleus motorius dorsalis nervi vagi (Figs. 1 G-K, 15, 17). The ventral part of the nucleus raphes inferior and the adjacent reticular formation (Figs. 1 F-K, 14) receive a low to medium density SP$_i$ innervation. Caudal to the obex a moderate density of fine varicosities is present in the dorsomedial part of the nucleus of the dorsal funiculus (Fig. 1 J, K). From here SP$_i$ fibers course ventrally into the nucleus of the solitary tract.

In the *spinal cord* SP$_i$ cell bodies are observed in the spinal ganglia (Fig. 16). In the spinal grey no SP$_i$ neurons are found. A narrow strip of thin to fairly thick varicose SP$_i$ fibers in the dorsal root enters the dorsalmost and lateral parts of the dorsal horn, where an extensive field of terminal structures is found in areas I and II of the spinal grey matter (after Kusuma et al. 1979; see Figs. 1 L-O, 18). Some of the dorsal root fibers can be traced to the dorsolateral part of area X, which surrounds the central canal; here a second, less densely innervated, terminal field is seen. In the ventral horn some sparse varicosities are found. Hardly any SP$_i$ fibers are present in the spinal funiculi.



Distribution of [Leu]enkephalin- and [Met]enkephalin-immunoreactive cell bodies, fibers and terminals in the brain stem and spinal cord.

Only few significant differences in the distribution of [Leu]enkephalin- (L-ENK$_i$; Fig. 19) and [Met]enkephalin- immunoreactivity (M-ENK$_i$; Fig. 20) were found, apart from the number of immunofluorescent cell bodies. A larger amount of L-ENK$_i$ cell bodies is present in the brain stem than M-ENK$_i$ neurons, whereas fiber densities are generally greater in the M-ENK$_i$ series.

In the caudal part of the *diencephalon* L-ENK$_i$ (Fig. 19 A) and M-ENK$_i$ (Fig. 20 A) cell bodies are found in the nucleus ventralis hypothalami, the nucleus periventricularis hypothalami and the ventral part of the lateral hypothalamic area (see also Fig. 26). L-ENK$_i$ neurons in the hypothalamus form a large cell cluster. Only few short neuronal processes can be discerned in the transversal plane. In addition, few L- and M-ENK$_i$ neurons were present in the paraventricular organ of one of the colchicine-treated animals (Figs. 23, 24). L-ENK$_i$ fibers are found in the area around the commissura posterior, particularly dorsolaterally and ventrally (Fig. 19 A). This area comprises the nucleus posterodorsalis, the medial part of the nucleus lentiformis thalami pars extensa and the dorsal part of the nucleus rotundus. In the hypothalamus a medium-density of L-ENK$_i$ varicosities is found in the nucleus periventricularis hypothalami (Figs. 19 A, 20 A, 25) just dorsal and lateral to the paraventricular organ. The L-ENK$_i$ and M-ENK$_i$ innervation of the paraventricular organ is shown in Figs. 23 and 24. In other parts of the periventricular hypothalamic nucleus and throughout the ventral and lateral hypothalamus a low density of L-ENK$_i$ varicosities is found, except for the lateral part of the nucleus ventralis hypothalami, which receives a high density innervation consisting of very fine varicosities. A comparable M-ENK$_i$ innervation of the caudal part of the diencephalon is found (Fig. 20 A).

In the *mesencephalon* a few L-ENK$_i$ cell bodies are found in the laminar nucleus of the torus semicircularis (Fig. 19 B), in the ventral part of the rostral tegmentum mesencephali and in the griseum centrale, just lateral to the dorsal part of the oculomotor nucleus. The latter cell group is especially abundant in M-ENK series (Fig. 20 C). Fine to medium-sized varicosities are present in the laminar nucleus of the torus semicircularis and other parts of the periventricular grey, including parts of the stratum griseum periventriculare of the tectum mesencephali (Fig. 19 B). A low density of the L-ENK$_i$ varicosities occurs in the nucleus profundus mesencephali and ventrolateral parts of the midbrain tegmentum. In M-ENK series a high density of M-ENK$_i$ fibers is found in the griseum centrale surrounding the aqueductus cerebri, particularly dorsolaterally. The more caudal part of the midbrain tegmentum, including the substantia nigra is less densely innervated (Fig. 20 C).

At the *isthmic* level (Figs. 19 C, 20 D) L-ENK$_i$ cell bodies are found in the nucleus reticularis superior pars lateralis. An ENK$_i$ innervation is found especially in the parabrachial region (Fig. 19 C), in high density in M-ENK series (Fig. 20 D). In this parabrachial region the enkephalinergic innervation broadly overlaps with the substance P innervation. In the periventricular zone a low density of L-ENK$_i$ varicosities (Fig. 19 C) is seen, and a more abundant M-ENK$_i$ innervation (Fig. 20 D). Other nuclei that receive a L-ENK and M-ENK innervation are the nucleus raphes superior and the adjacent reticular formation, and the ventral part of the nucleus interpeduncularis (Figs. 19 C, 20 C, D). In the granular layer of the cerebellum a medium density of rather thick varicosities is present as well as a few thin varicose fibers (Figs. 19 C, 20 D, 28).

In the *rhombencephalon* at the level of the motor nucleus of the trigeminal nerve (Fig. 19 D) L-ENK$_i$ cell bodies are present in and around the descending nucleus of the trigeminal nerve (Fig. 32), ventral to the motor nucleus of the trigeminal nerve and scattered in the ventro-lateral part of the rostral rhombencephalic reticular formation (see also Fig. 27). Less M-ENK$_i$ neurons are observed in this part of the rhombencephalon (Fig. 20 E). More caudally, particularly L-ENK$_i$ cells are found in the lower brain stem. These neurons are present in and around the rostral part of the descending vestibular nucleus (Fig. 19 E), in the ventral part of the nucleus tractus solitarii as well as ventral to this nucleus (Fig. 19 F, G) and in the ventrolateral part of the nucleus reticularis inferior (Fig. 19 F). Less M-ENK$_i$ cells are found in the caudal rhombencephalon (see Fig.

20 G-I).

At the level of the trigeminal nerve root (Fig. 19 D) a medium density of very fine to fine L-ENK$_i$ varicosities is present ventral to and within the nucleus descendens nervi trigemini. Furthermore, a distinct L-ENK innervation of the motor nucleus of the trigeminal nerve is found (Figs. 19 D, 31). Both areas receive a M-ENK innervation of higher density (Fig. 20 E). The periventricular grey, especially at the ventrolateral border of the fourth ventricle, contains a medium to low density of L-ENK$_i$ and M-ENK$_i$ varicosities.

In the medulla oblongata a dense plexus of L-ENK$_i$ and M-ENK$_i$ fibers with very thin to fairly thick varicosities is found in the ventral part of the descending trigeminal tract and, more medially, at the level of the nucleus ambiguus (Figs. 19 E, 20 G, 33). The vestibular nuclear complex (Figs. 19 D, E, 20 E-G, 30), particularly the descending vestibular nucleus, contains a number of fairly thick L-ENK$_i$ and M-ENK$_i$ varicosities, varying from low to medium density. In the reticular formation areas of low to medium density L-ENK- and M-ENK-immunoreactivity (Figs. 19 E, F, 20 G-I) are present, particularly in the ventral part of the raphe and the lateral part of the reticular formation. In this area the highest density is found in the nucleus raphes inferior (see Fig. 29). In the dorsocaudal part of the medulla oblongata medium density L- and M-ENK$_i$ terminal structures are present in the medial part of the nucleus of the solitary tract and the dorsal motor nucleus of the vagus nerve (Figs. 19 F, G, 20 I, 34).

In the *spinal cord* (Figs. 19 H-K, 20 J-M, 35, 36) L-ENK$_i$ and M-ENK$_i$ structures are present in the white and grey matter. In the white matter L-ENK$_i$ varicosities are present at all levels of the spinal cord, particularly in the tract of Lissauer and the dorsal part of the lateral funiculus (Figs. 19 H-K, 20 J-M, 36). A less dense L- and M-ENK$_i$ fiber plexus is found in the remainder of the lateral funiculus. No L-ENK$_i$ or M-ENK$_i$ fibers are present in the dorsal or ventral funiculus. In the grey matter very fine to medium-sized L- and M-ENK$_i$ varicosities are predominantly found in the dorsal horn, particularly in the region comparable to areas I and II (see Fig. 35), but also in area X which directly surrounds the central canal. Particularly the high density of the L- and M-ENK$_i$ innervation of area X at lumbar levels (Figs. 19 J, 20 L) should be noted. A medium density L- and M-ENK$_i$ innervation is found in parts of the intermediate zone (areas V/VI and VII/VIII after Kusuma et al. 1979). Only a sparse innervation of the ventral horn is present (see Fig. 36). No L- or M-ENK$_i$ cell bodies are found in the spinal cord or dorsal root ganglia.

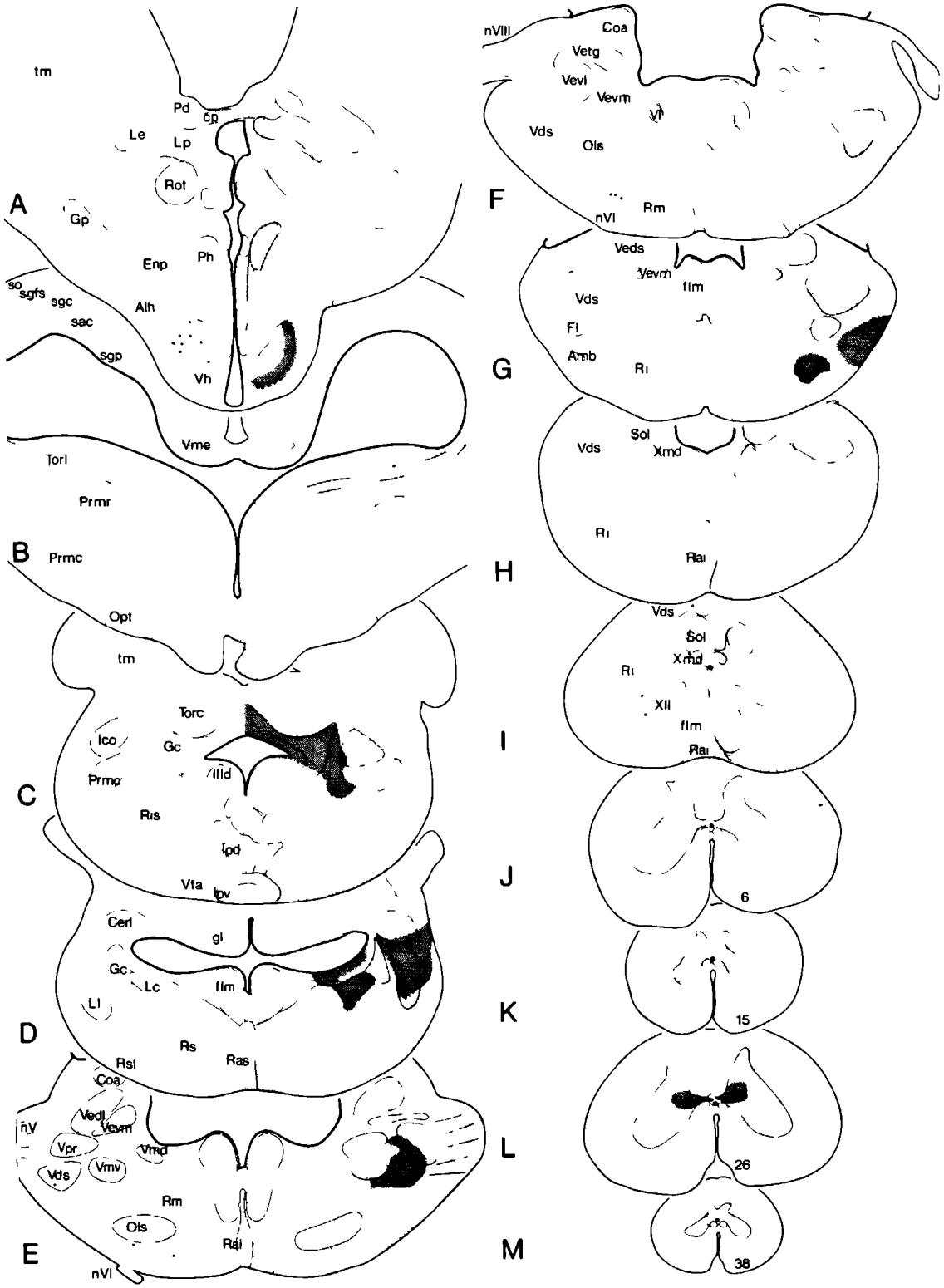
Discussion

The present data indicate a rather widespread distribution of substance P-like immunoreactivity in the brain stem and spinal cord of a nonmammalian vertebrate, the lizard *Varanus exanthematicus*. This distribution is comparable to that in other vertebrates such as amphibians (Inagaki et al. 1981; Taban and Cathieni 1983), turtles (Reiner et al. 1984) and mammals (see e.g. Cuello and Kanazawa 1978; Ljungdahl et al. 1978a, b). It should be noted that the molecular structure of the SP-like material in reptiles and other nonmammalian vertebrates is so far unknown; interphyletic variations may be present (see e.g. Barrington 1982), and a number of other tachykinins which share a common C-terminal amino acid sequence with the undecapeptide SP, as physalaemin and eledoisin (Erspamer 1981; Nakajima 1981) have been extracted from nonmammalian tissues. The distribution pattern and radioimmunoassay studies (see Reiner et al. 1984), however, strongly suggest that the SP-like tachykinin that predominates in the reptilian nervous system is either highly similar or identical to the mammalian SP.

The present results obtained in the brain stem and spinal cord of the lizard *Varanus exanthematicus*, by applying antibodies directed against [Leu]- or [Met]enkephalin should also be viewed with some caution. The term "enkephalin-immunoreactive" defines the nature of the immunoreaction on the basis of antibody characterization but not necessarily the nature of the tissue antigen responsible for the reaction (Cuello 1983). Other related opioids like e.g. dynorphin might be responsible for at least part of the immunoreactivity demonstrated. The close resemblance between L-ENK and M-ENK immunoreactivity is not unexpected in the light of recent studies (see Akil et al. 1984 for review), indicating that both opioid peptides are derived from the same precursor molecule, proenkephalin.

The distribution of SP immunoreactivity in the brain stem and spinal cord of the lizard *Varanus exanthematicus* has been summarized in Fig. 37. The SP-containing striatotegmental pathway, already demonstrated in turtles (Brauth et al. 1983; Reiner et al. 1984) and the crocodilian *Caiman crocodilus* (Brauth et al. 1983) is also well developed in the lizard *Varanus exanthematicus*. This pathway, probably arising in SP$_i$ cell bodies in the striatum

Fig. 20 A-M. Schematic representation of M-ENK immunoreactivity as observed in representative transversal sections of the brain stem and spinal cord of colchicine-treated lizards (*Varanus exanthematicus*). At the right, the density of M-ENK$_i$ fibers and varicosities is indicated, subjectively classified into five categories: *1.* no or hardly any fluorescence; *2.* low density; *3.* medium density; *4.* high density; *5.* very high density. At the left dots indicate M-ENK-immunofluorescent cell bodies in each section. Numbers within the confines of Fig. 20 J-M indicate spinal levels of the pictured sections. (Bar: 0,5 mm)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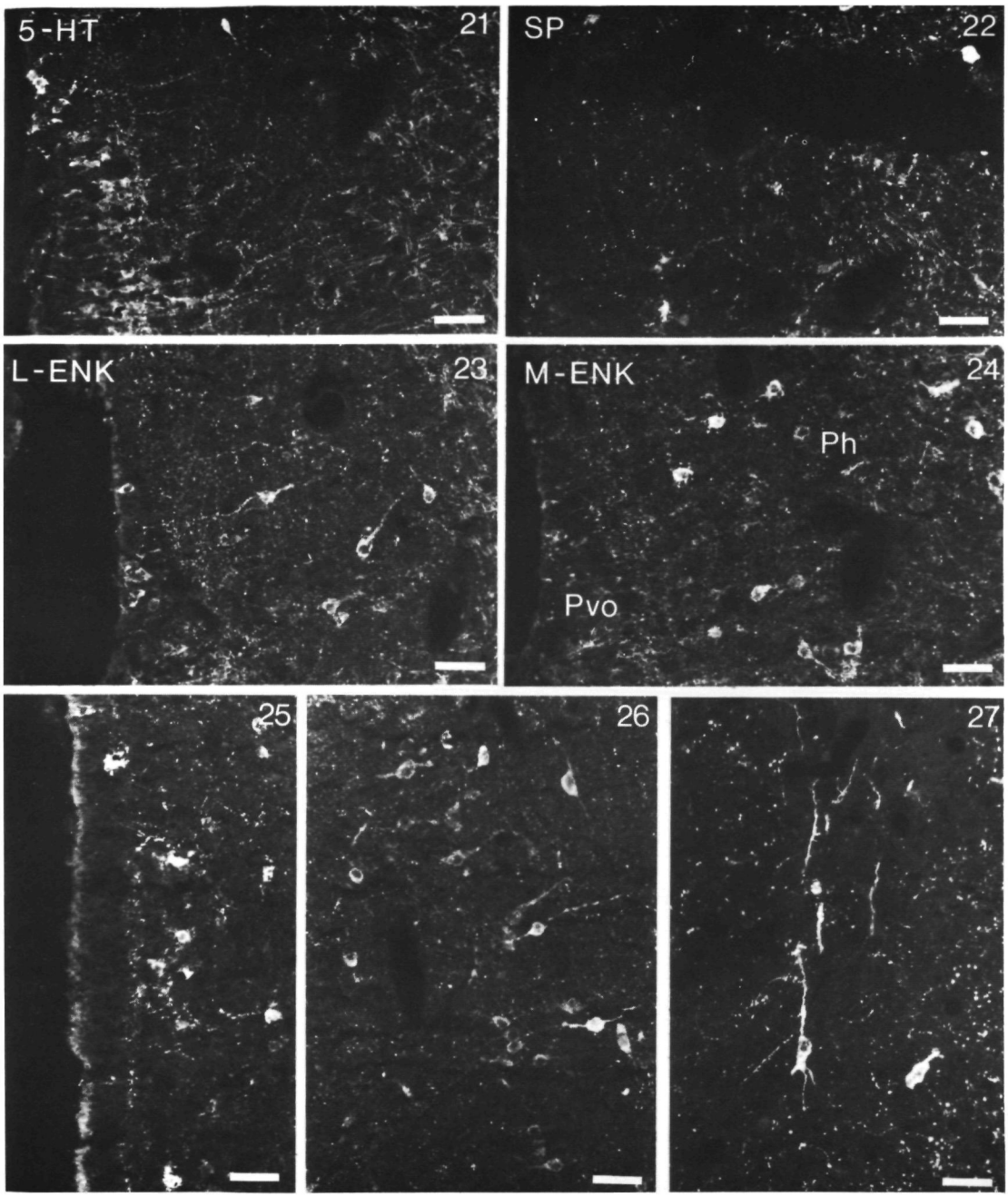

Figs. 21-24. Diencephalon. Four adjacent sections through the paraventricular organ (Pvo) and the adjacent part of the nucleus periventricularis hypothalami (Ph), stained, respectively, for serotonin (5-HT) which heavily labels most cells of the paraventricular organ, substance P (SP), [Leu]enkephalin (L-ENK) and [Met]enkephalin (M-ENK). Note the presence of L- and M-ENK$_i$ neurons and a few SP$_i$ neurons in Ph. In Pvo a few L- and M-ENK$_i$ cells are visible, as observed in only one of the colchicine-treated animals; these neurons have a more medial position than the numerous 5-HT$_i$ cells in the Pvo. (Bar: 35 μm) **Fig. 25.** Diencephalon. L-ENK$_i$ cell bodies and varicosities in the ventral part of Ph. (Bar: 35 μm) **Fig. 26.** Diencephalon. Numerous M-ENK$_i$ neurons in the nucleus ventralis hypothalami. (Bar: 35 μm) **Fig. 27.** Rostral rhombencephalon. Some L-ENK$_i$ cell bodies and very fine to fairly thick L-ENK$_i$ varicosities in the nucleus reticularis medius. (Bar: 30 μm)

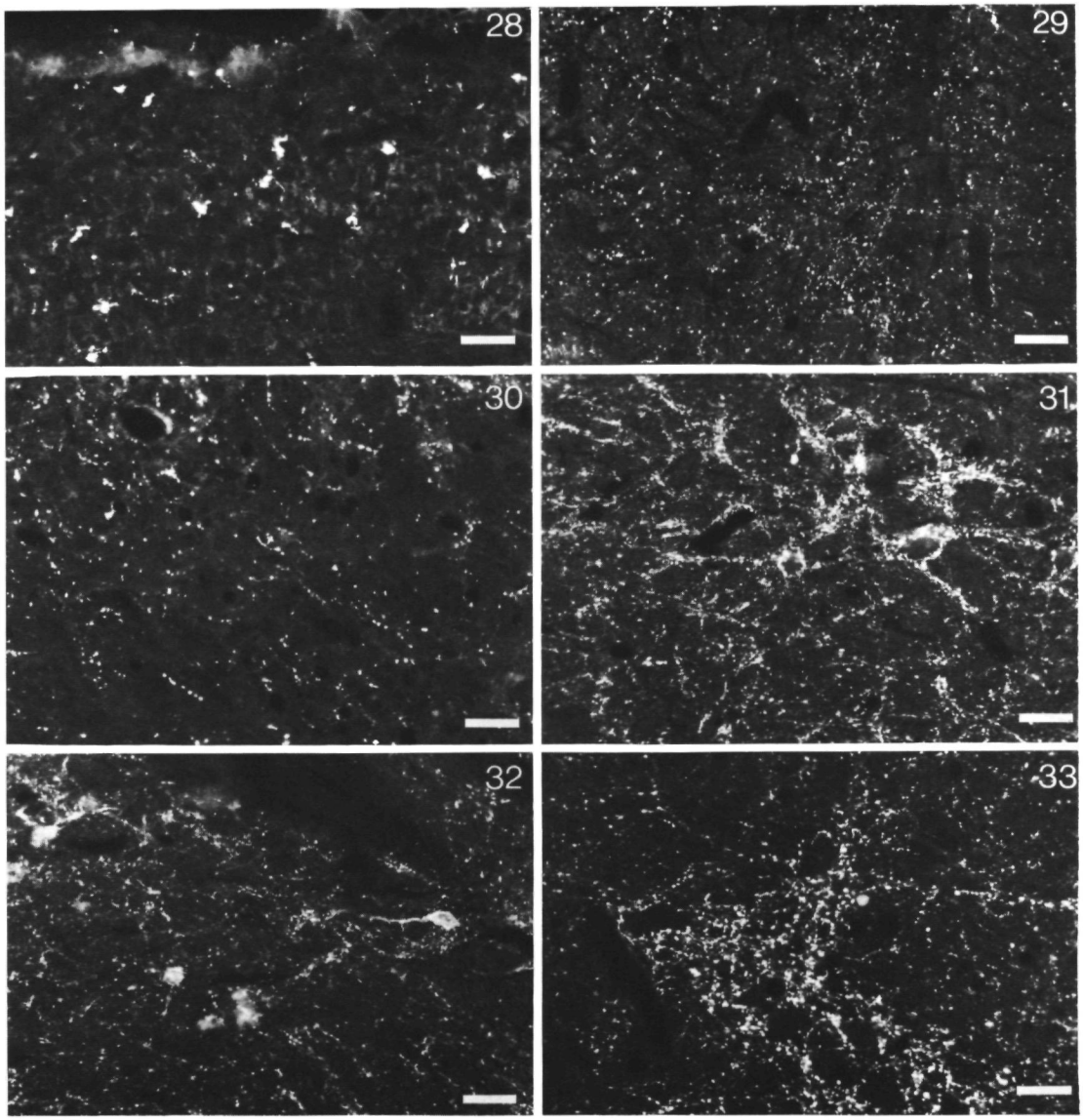

Fig. 28. Cerebellum. Mainly very thick but also some very fine M-ENK$_i$ varicosities in the granular layer. (Bar: 35 μm) **Fig. 29.** Caudal rhombencephalon. Mainly fine and very fine L-ENK$_i$ varicosities innervating the ventral part of the nucleus raphes inferior. (Bar: 35 μm) **Fig. 30.** Rostral rhombencephalon. Medium-sized and fine L-ENK$_i$ varicosities in the nucleus vestibularis dorsolateralis. (Bar: 35 μm) **Fig. 31.** Rostral rhombencephalon. High density L-ENK$_i$ innervation of the nucleus motorius nervi trigemini; note, that perikarya and dendrites are completely covered by fine and very fine L-ENK$_i$ varicosities. (Bar: 35 μm) **Fig. 32.** Rostral rhombencephalon. L-ENK$_i$ cell bodies, fine to very fine varicosities and fibers, present in the nucleus descendens nervi trigemini. (Bar: 35 μm) **Fig. 33.** Caudal rhombencephalon. Very high density L-ENK innervation of the nucleus ambiguus by very fine to medium-sized L-ENK$_i$ varicosities. (Bar: 35 μm)

(Wolters and ten Donkelaar, unpublished observations), can be traced via the lateral forebrain bundle to the mesencephalic tegmentum (see Fig. 1) in keeping with anterograde and retrograde tracer studies in lizards (Hoogland 1977; Voneida and Sligar 1979; ten Donkelaar and de Boer-van Huizen 1981a). The SP$_i$ striatotegmental pathway terminates for the greater part ventro-laterally to the group of catecholaminergic (presumably dopaminergic) neurons defined as substantia nigra (Wolters et al. 1984). This ventrolateral area with its extensive SP innervation and projections to the tectum mesencephali and lower brain stem (see ten Donkelaar and de Boer-van Huizen 1981a)

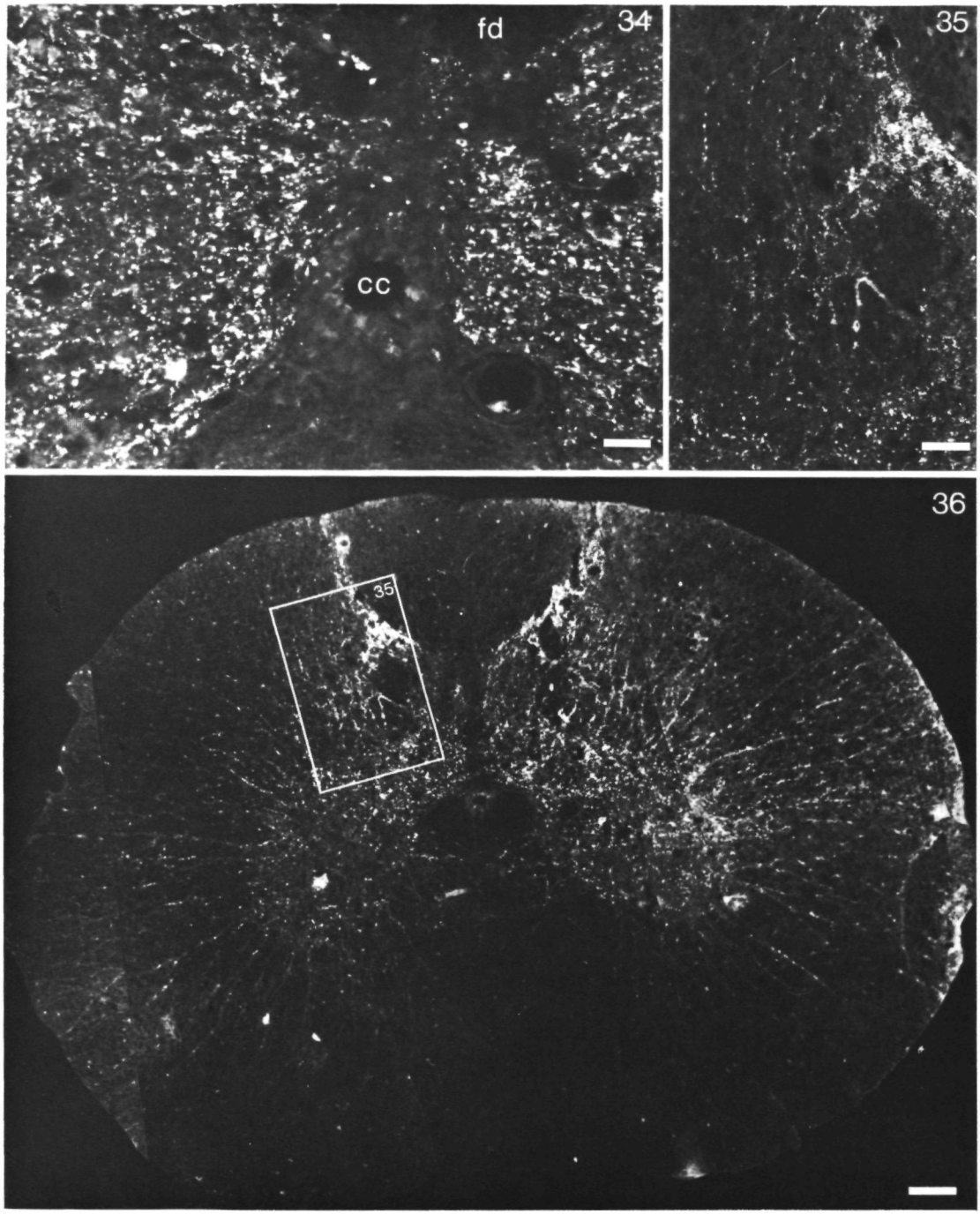

Fig. 34. Cervical intumescence of the spinal cord. L-ENK innervation of area X, dorsal and lateral to the central canal (cc) by numerous very fine to medium-sized L-ENK$_i$ varicosities. (Bar: 25 μm) **Fig. 35.** Thoracic level of the spinal cord. Detail of Fig. 36. L-ENK$_i$ fibers and varicosities, innervating the dorsal horn and intermediate zone. (Bar: 35 μm) **Fig. 36.** Thoracic level of the spinal cord. General view of L-ENK immunoreactivity. Dense innervation of areas I and II in the dorsal horn, medium density innervation of areas X, V/VI and VII/VIII, and sparse varicosities in the ventral horn. Also note the presence of varicosities on long neuronal processes penetrating deeply in the white matter. In the pictured section only very few cross-sectioned L-ENK$_i$ fibers can be discerned. (Bar: 85 μm)

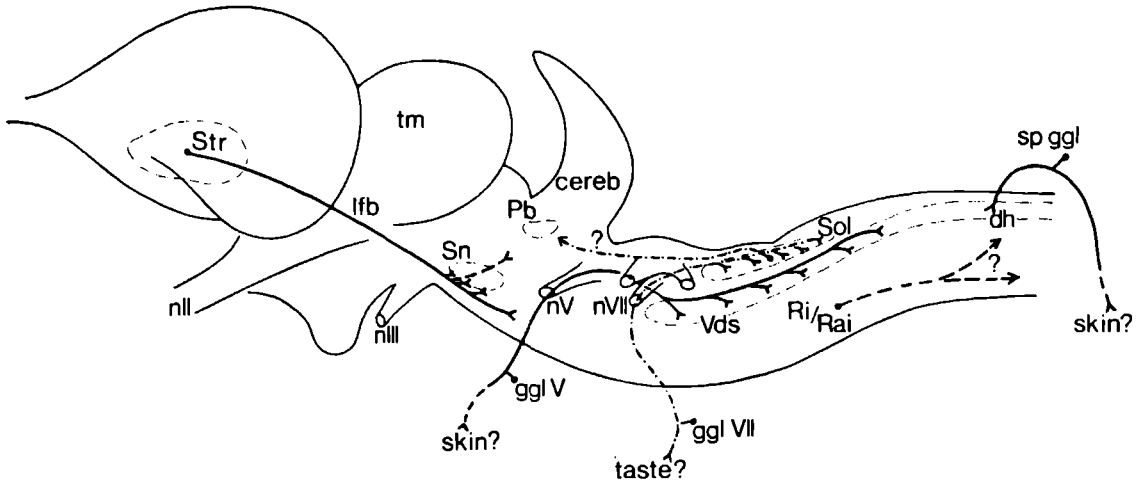

Fig. 37. Lateral view of brain stem and upper part of the cervical spinal cord of the lizard *Varanus exanthematicus*, in which the main substance P-containing projections have been summarized. Question marks indicate pathways for the existence of which evidence was gathered from this and many other studies, as discussed in the text.

presumably represents the reptilian homologue of the mammalian substantia nigra pars reticulata. A medial extension of t-fb--df-he striatotegmental tract terminates in the catecholaminergic ventral tegmental area (see Wolters et al. 1984). The striatotegmental pathway continues to the caudal midbrain and the parabrachial region. Similar observations have been made by Reiner et al.(1984). A SP-containing striatonigral pathway has also been demonstrated in the pigeon *Columba livia* (Reiner et al. 1983) and various mammals (see e.g. Brownstein et al. 1976, 1977; Cuello and K!-b7- zawa 1978; Hong et al. 1977; Kanazawa et al. 1977; Ljungdahl et al. 1978a). So far no SP-positive habenulo-interpeduncular pathway (fasciculus retroflexus) as shown in mammals (see e.g. Cuello et al. 1978; Emson et al. 1977; Hong et al. 1976; Mroz et al. 1976), has been demonstrated in reptiles (Engbretson et al. 1982; Reiner et al. 1984; the present study). SP_i fibers in and close to the pineal gland may be related to the parietal eye (see also Engbretson et al. 1982).

Apart from the cell masses in the tegmentum mesencephali discussed above, particularly the following structures in the brain stem and spinal cord receive an extensive SP innervation: the nucleus of the solitary tract, the descending nucleus of the trigeminal nerve and the dorsal horn of the spinal cord. The SP projections to the nucleus of the solitary tract, presumably related to taste or visceral information, follow the course of central projections of the VII^{th}, IX^{th} and X^{th} cranial nerves in the brain stem (see Barbas-Henry 1982; Barbas-Henry and Lohman 1984). The cells of origin of such projections are to be expected in the ganglia of these cranial nerves. The SP_i cell bodies in the nucleus of the solitary tract may form part of the cells of origin of ascending taste pathways to the parabrachial region and beyond (see ten Donkelaar and de Boer-van Huizen 1981b). Both the nucleus of the solitary tract which contains $L\text{-}ENK_i$ cell bodies and the parabrachial region receive a dense ENK innervation. This situation resembles the pattern found in mammals. In the rat several neuropeptides including substance P and [Met]enkephalin, are present in projection neurons at all levels in visceral and taste pathways as the nodose ganglion, the nucleus of the solitary tract and the parabrachial nucleus (Mantyh and Hunt 1984).

The SP_i projections to the descending nucleus of the trigeminal nerve and the dorsal horn have their cells of origin in the trigeminal ganglion and spinal ganglia, respectively. The SP innervation of the caudal part of the descending trigeminal nucleus (see e.g. Fig. 1 K) resembles the termination pattern in the dorsal horn of the spinal cord. In the descending nucleus of the trigeminal nerve of mammals (see e.g. Del Fiacco and Cuello 1980) SP_i fibers innervate the superficial layers of this nucleus.

The SP innervation of the spinal cord is concentrated in the dorsal horn, mainly in areas I and II (after Kusuma et al. 1979), but also around the central canal (area X and adjacent parts of area V-VI) a distinct SP innervation is found. Only a sparse innervation of the ventral horn is found in the lizard *Varanus exanthematicus*. The SP innervation of the superficial parts of the dorsal horn of the spinal cord is common for all vertebrates studied so far: the stingray *Dasyatis sabina* (Ritchie and Leonard 1983), the newt *Triturus cristatus* (Taban and Cathieni 1983), the frogs *Rana esculenta* (Lorez and Kemali 1981) and *Rana catesbiana* (Inagaki et al. 1981), reptiles (Reiner et al. 1984; the present study), birds (Bayon et al. 1980; LaValley and Ho 1983) and mammals, e.g. the opossum *Didelphis virginiana* (DiTirro et al. 1981), the rat (Hökfelt et al. 1977b; Cuello and Kanazawa 1978; Ljungdahl et al. 1978a,

b; Gibson et al. 1981; Hunt 1983), the cat (Hökfelt et al. 1975a; Tessler et al. 1981) and primates including man (Cuello et al. 1976; LaMotte and DeLanerolle 1981; DiFiglia et al. 1982; Charnay et al. 1983). Ligation and transection (dorsal rhizotomy) studies (Takahashi and Otsuka 1975; Hökfelt et al. 1975a; Barber et al. 1979; Tessler et al. 1981) have shown that SP in the superficial layers of the dorsal horn is largely present in nerve terminals representing central branches of the primary sensory neurons in the spinal ganglia. A smaller contribution is derived from SP_i neurons in the spinal grey including the superficial layers of the dorsal horn (Ljungdahl et al. 1978a; Barber et al. 1979; Hunt 1983). Such neurons, also reported in the frog *Rana esculenta* (Lorez and Kemali 1981) have not been demonstrated by us in the lizard *Varanus exanthematicus*.

In mammals, the SP innervation of the ventral horn is probably supraspinal in origin since after thoracic transections of the spinal cord almost all SP_i fibers disappear in the ventral horn below the lesion (see e.g. Kanazawa et al. 1979; Ljungdahl et al. 1978a). With combined retrograde tracing-immunohistochemical techniques (see e.g. Bowker et al. 1981, 1982, 1983; Hökfelt et al. 1982) supraspinal SP projections were found to arise in the raphe nuclei and adjacent ventrolateral reticular formation of the medulla oblongata. In reptiles the ventral horn receives only a very sparse SP innervation (Reiner et al. 1984; the present study). A brain stem origin of this innervation is possible, presumably from the SP_i cells in the nucleus reticularis inferior and nucleus raphes inferior. SP_i neurons in the periventricular region of the rostral mesencephalon may also participate in this projection since here spinal projections arise (ten Donkelaar et al. 1980). In the rat SP containing neurons in the rostral periaqueductal grey of the mesencephalon are known to project to the spinal cord (see Hökfelt et al. 1982).

The distribution of [Leu]- and [Met]enkephalin in the brain stem and spinal cord of the lizard *Varanus exanthematicus* is less impressive than that of substance P. In the present study enkephalinergic cell bodies were found particularly in the caudal hypothalamus in keeping with data in other reptiles (Naik et al. 1981; Reiner 1983; Brauth 1984). Less extensive populations of enkephalinergic cell bodies are present in the vestibular nuclear complex, the nucleus of the solitary tract and in and around the descending nucleus of the trigeminal nerve. Enkephalins are likely to be present in the following pathways: 1) efferent projections of the striatum via the lateral forebrain bundle (Naik et al. 1981; Reiner 1983; Brauth 1984; Wolters and ten Donkelaar, unpublished observations in *Varanus exanthematicus*), 2) central taste and/or visceral pathways: neurons in the nucleus of the solitary tract are known to project to the parabrachial region (ten Donkelaar and de Boer-van Huizen 1981b), and 3) descending pathways to the spinal cord: enkephalinergic fibers are present in the lateral funiculus and ENK_i cell bodies are found in the reticular formation, particularly in the nucleus reticularis inferior, known to project to the spinal cord via the lateral funiculus (ten Donkelaar et al. 1980; Wolters et al. 1982; Newman et al. 1983). However, combined retrograde tracing-immunohistochemical techniques are necessary to demonstrate the existence of ENK_i supraspinal projections in nonmammalian vertebrates.

In the spinal cord ENK_i terminal structures are especially found in the superficial layers of the dorsal horn (areas I and II after Kusuma et al. 1979) and around the central canal (area X and adjacent parts of areas V-VI and VII-VIII). The ventral horn including the motoneuron area (area IX) receives a relatively sparse ENK_i innervation, particularly by L-ENK. An ENK_i innervation of the superficial layers of the dorsal horn seems to be common for vertebrates. Immunohistochemical studies in the frog *Rana esculenta* (Lorez and Kemali 1981), reptiles (Naik et al. 1981; Reiner 1983; the present study), the bird *Gallus domesticus* (LaValley and Ho 1983 and mammals, e.g. the opossum *Didelphis virginiana* (DiTirro et al. 1981), the rat (Hökfelt et al. 1977a; Simantov et al. 1977; Sar et al. 1978; Finley et al. 1981; Gibson et al. 1981; Hunt 1983; Khachaturian et al. 1983), the cat (Glazer and Basbaum 1980, 1981) and primates including man (Haber and Elde 1982; LaMotte and DeLanerolle 1981; Charnay et al. 1984), all demonstrate a dense ENK_i innervation of the superficial layers of the dorsal horn. This innervation is presumably largely intrinsic to the cord, i.e. arising from local circuit neurons. In mammals after pharmacological manipulation (injection of colchicine directly into the cord) ENK_i cell bodies could be demonstrated in all layers of the dorsal horn, but particularly in layers I and II, which suggests the occurrence of local spinal enkephalin neurons (Hökfelt et al. 1977a; Sar et al. 1978; Uhl et al. 1979; Glazer and Basbaum 1980, 1981; Hunt et al. 1981a, b; Hunt 1983). ENK_i cell bodies in the dorsal horn of the spinal cord have also been demonstrated in nonmammalian vertebrates such as the frog *Rana esculenta* (Lorez and Kemali 1981), the lizard *Anolis carolinensis*(Naik et al. 1981 Figs. 17, 42) and the turtles *Chrysemys picta* and *Pseudemys scripta* (Reiner 1983). In the present study, however, no ENK_i cell bodies could be demonstrated in the spinal grey of *Varanus exanthematicus*.

The widespread distribution of ENK_i fibers in the lateral funiculus of the spinal cord suggests in addition a supraspinal contribution. Possibly ENK_i cell bodies in the nucleus reticularis inferior are the source of this component. With combined retrograde tracing-immunohistochemical techniques enkephalin-containing neurons were localized in the rat in the nucleus raphe magnus and ventral parts of the nucleus reticularis gigantocellularis (Hökfelt et al. 1979, 1982; Bowker et al. 1982, 1983).

Thus, it appears that in many respects the ENK_i distribution in the brain stem and spinal cord of the lizard *Varanus exanthematicus* is comparable to that in mammals (see e.g. Simantov et al. 1977; Sar et al. 1978; Finley et al. 1981; Uhl et al. 1981; Khachaturian et al. 1983). In mammals ENK_i fibers and terminals are present in high concentration in areas related to pain and analgesia (Hökfelt et al. 1977a, c; Simantov et al. 1977). This distribution seems to follow consistently the distribution of opioid receptors, as judged by either binding or autoradiographic techniques (see Atweh and Kuhar 1983 for review). High levels of opioid receptor binding sites have been reported (Pert et al. 1974) in the CNS of various vertebrates including dog fishes, the toad *Bufo marinus*, the turtle *Chrysemys picta* and several mammalian species. Although the regional distribution of opioid receptors was not analysed in all vertebrates studied (e.g. not in *Chrysemys*) by Pert et al. (1974) opioid peptides are presumably an ubiquitous feature of the vertebrate CNS, particularly of the dorsal horn of the spinal cord.

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6

COLLATERALIZATION OF DESCENDING PATHWAYS FROM THE BRAIN STEM TO THE SPINAL CORD IN A LIZARD, VARANUS EXANTHEMATICUS, AS DEMONSTRATED WITH THE MULTIPLE FLUORESCENT RETROGRADE TRACER TECHNIQUE

Abstract — With the multiple fluorescent retrograde tracer technique, the collateralization in the spinal cord of descending supraspinal pathways was studied in a lizard, *Varanus exanthematicus* "Fast Blue" (FB) gels were implanted unilaterally in the spinal grey matter of the cervical enlargement, whereas "Nuclear Yellow" (NY) gels were implanted, ipsilaterally, in all spinal funiculi of the lumbar enlargement or in midthoracic spinal segments of the cord, in two consecutive series of experiments, respectively All brain stem nuclei known to project to the spinal cord in reptiles, were found to give rise to branching axons that may influence widely separated levels of the spinal cord The amount of double-labeled FB-NY cells varied, in the 23 brain stem nuclei studied, from 0% to 50% of the number of neurons projecting to the cervical enlargement Highly collateralizing projections were found to arise from the ipsilateral nucleus periventricularis hypothalami, the nucleus reticularis superior, the nucleus reticularis superior pars lateralis, the contralateral nucleus princeps nervi trigemini, the contralateral nuclei vestibulares ventromedialis and descendens, the nucleus reticularis inferior pars ventralis, and the nucleus raphes inferior. Extensive brain stem projections for the greater part directed to cervical and high thoracic spinal levels originate from the area lateralis hypothalami, the ipsilateral nucleus interstitialis of the fasciculus longitudinalis medialis, the nucleus of the fasciculus longitudinalis medialis, the contralateral nucleus ruber, the contralateral nucleus cerebellaris medialis, the nucleus vestibularis ventrolateralis, and from the nucleus tractus solitarii Projections preferentially directed to midthoracic or lower levels of the spinal cord, were found to arise from the ipsilateral locus coeruleus, the contralateral nucleus reticularis superior pars lateralis, the nucleus reticularis inferior pars ventralis, the nucleus reticularis inferior, and the nucleus raphes inferior In contrast to findings in mammals, e.g. with respect to the red nucleus, the nucleus vestibularis ventrolateralis, and certain parts of the reticular formation, none of the projections studied in *Varanus exanthematicus* displayed a clear-cut somatotopic organization

In general two different patterns of collateralization could be distinguished 1) the amount of spinal collaterals is approximately constant beyond the midthoracic spinal level; 2) the amount of spinal collaterals is gradually decreasing caudalward The latter pattern is found in the projections from e.g the ipsilateral nucleus periventricularis hypothalami, the nucleus interstitialis of the fasciculus longitudinalis medialis, the nucleus ruber, the nucleus cerebellaris medialis, the nucleus princeps nervi trigemini, the nucleus vestibularis ventrolateralis, the ipsilateral nucleus vestibularis ventromedialis, the nucleus vestibularis descendens, the nucleus prepositus hypoglossi, the nucleus tractus solitarii, the nucleus reticularis inferior pars lateralis, the ipsilateral nucleus reticularis inferior, and the nucleus raphes inferior These data, however, do not allow conclusions with respect to the specificity (focussed, or global information) of the various descending supraspinal pathways

Introduction

Since the last decade the use of several experimental neuroanatomical techniques has enlarged our knowledge about the organization of descending brain stem pathways throughout vertebrates. Especially studies with anterograde (Basbaum et al. 1978; Holstege et al 1979; Holstege and Kuypers

1982; Goode et al. 1980; Martin et al. 1979, 1981c, 1982) and retrograde (see e.g. Kuypers and Maisky 1975, 1977; Crutcher et al. 1978; Tohyama et al. 1979a,b; ten Donkelaar et al. 1980, 1981; Hayes and Rustioni 1981; Smeets and Timerick 1981; Woodson and Künzle 1982; Cabot et al. 1982; Newman et al. 1983) tracer techniques have provided a wealth of new data on origin, course and site of termination of these pathways.

In various mammals a somatotopic pattern within the projections to the spinal cord has been demonstrated for several brain stem nuclei, such as the red nucleus (Pompeiano and Brodal 1957a; Nyberg-Hansen and Brodal 1964; Miller and Strominger 1973; Murray et al. 1976; Castiglioni et al. 1978; Kneisley et al. 1978; Murray and Gurule 1979; Zemlan et al. 1979; Hayes and Rustioni 1981; Padel et al. 1981; Watkins et al. 1981; Huisman et al. 1981, 1982) and the lateral vestibular nucleus (Pompeiano and Brodal 1957b; Nyberg-Hansen and Mascitti 1964; Kneisley et al. 1978; Zemlan et al 1979). Even within the reticular formation some degree of somatotopic organization in the projections to the spinal cord has been found (see e.g. Peterson et al. 1975, 1979; Zemlan et al. 1979; Martin et al. 1981c).

Electrophysiological studies showed the existence of a considerable amount of neurons, present in the red nucleus (Shinoda et al. 1977), the vestibular nuclear complex (Abzug et al. 1973, 1974) and in the reticular formation (Peterson et al. 1975, 1979; Peterson 1980), that give rise to branching axons, which project to widely separate levels of the spinal cord as the cervical and lumbar enlargements. Although the phenomenon of axon collateralization has already been demonstrated with the Golgi technique by Cajal (1909; see also Scheibel and Scheibel 1958), only recently appropriate neuroanatomical techniques to study axonal collateralization became available: 1) the double retrograde tracer technique with horseradish peroxidase (HRP) and tritiated, enzymatically inactive HRP (^{3}H-apo-HRP) as tracers (Hayes and Rustioni 1979, 1981); 2) the HRP - iron dextran technique (Olsson and Kristensson 1978; Cesaro et al. 1979); 3) the multiple retrograde fluorescent tracer technique (Kuypers et al. 1980; Kuypers and Huisman 1984). In the latter technique several fluorescent substances (as e.g. "Fast Blue" and "Nuclear Yellow") can be used as tracers, which, after retrograde transport through divergent axon collaterals, reach the same parent cell body where they can be visualized independently by means of fluorescence microscopy.

In mammals the fluorescent retrograde double labeling technique has been used successfully in studies on the collateralization of descending brain stem pathways (Huisman et al. 1981, 1982, 1984; Martin et al. 1981a,b).

In the present study the multiple retrograde fluorescent tracer technique has been applied to the descending pathways from the brain stem to the spinal cord in a nonmammalian vertebrate, the monitor lizard *Varanus exanthematicus*. Previous studies on descending pathways in this lizard (ten Donkelaar et al. 1980; Wolters et al. 1982, 1984, 1985a,b) form the background for a study approaching four issues: 1) Do the descending brain stem pathways give off collaterals that may influence widely separate levels of the spinal cord as the cervical and lumbar enlargements ? 2) Is there evidence for a somatotopic pattern within brain stem projections to the spinal cord ? 3) Are there quantitative differences in the degree of collateralization of different descending brain stem pathways ? 4) Are there differences in the pattern of collateralization of the different descending pathways ?

Materials and techniques

The multiple retrograde fluorescent tracer technique as used in the present study

Several fluorescent substances with different characteristics, which are taken up by nerve endings or damaged nerve fibers, can be used as retrograde tracers. If two or more of them are injected in different parts of the nervous system, these tracers will be transported back to the parent cell bodies of the fibers by which they are taken up. In the case of collaterals to the various injection areas, the different tracers will be stored in the same perikaryon. Here they can be visualized independently, with the aid of a standard fluorescence microscope. If the tracers used have different excitation wavelengths (e.g. "True Blue" and Propidium Iodide), they must be viewed with different filter-mirror combinations, but if the tracers used are concentrated in different parts of the cell body and have different emission wavelengths (e.g. "Fast Blue" and "Nuclear Yellow", used in the present study, which are stored in the cytoplasm and the nucleus of the cell body, respectively), both tracers can be viewed at the same time with the same filter combination.

The aim of the present study was to demonstrate the existence of collaterals from brain stem neurons, projecting to widely separate levels of the spinal cord. Therefore, "Fast Blue" (FB) was applied unilaterally to the spinal grey of the cervical enlargement, and "Nuclear Yellow" (NY) to the lumbar enlargement (group I experiments) or to the mid-thoracic levels (group II experiments).

Since at the cervical cord level the applied tracer should be taken up only by nerve terminals, and not by damaged or even intact passing fibers (see discussion; Fig. 1), limiting the spread of the dye to the spinal grey matter is of utmost importance. This was obtained in several ways: a) by using dye-containing gels instead of injections of the dissolved tracer; b) by using FB, which remains fairly well restricted to

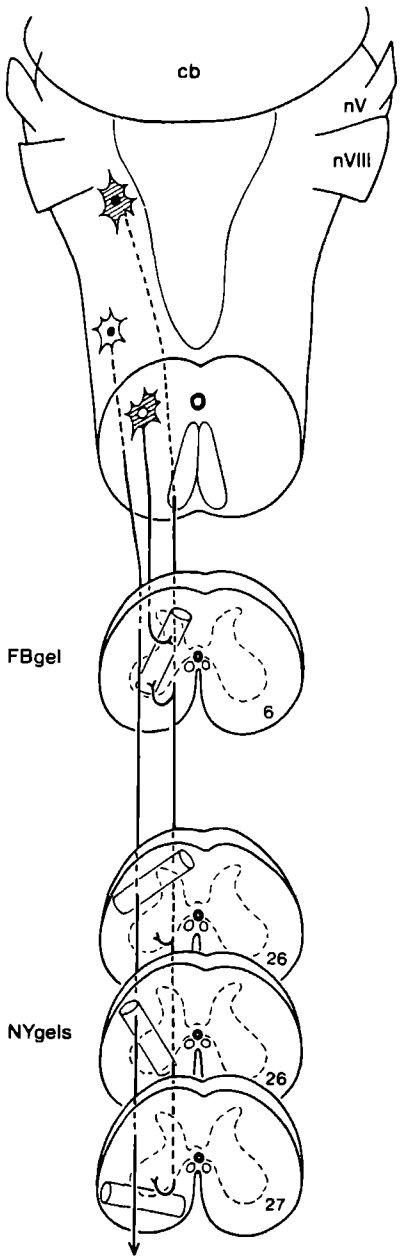


Fig. 1. Schematic view of the retrograde labeling of brain stem neurons following FB gel implantation in the cervical spinal grey and NY application to the fibers descending beyond lumbar or thoracic level. 1) Any FB labeled cell in the brain stem is projecting at least to the cervical intumescence, but may project to other spinal levels as well. 2) Any NY labeled cell in the brainstem is projecting to or beyond the level of the NY gel implantation, but may also project to higher levels of the cord, except for the cervical intumescence. 3) Any DL neuron is projecting to the cervical intumescence as well as to or beyond the level of the NY gel implantation, but moreover may project to other spinal levels.

the direct environment of the implantation site; c) by implanting not more than two FB gels, one caudal to the other, in order to minimize the risk of damaging passing fibers.

At lumbar (I) or thoracic (II) levels of the spinal cord NY gels were implanted ipsilaterally in all spinal funiculi (Fig. 1), thereby damaging as many fibers as possible, in order to maximize the number of fibers projecting to or passing beyond that level, that take up the dye. Furthermore, it should be noted that NY spreads much more easily into the surrounding tissue, even into the compact white matter, than FB (Figs. 2 A-B), and, considering our purposes, is therefore very suitable for use at the caudal implantation level.

The use of dye-containing gels has several advantages as compared to injections of the fluorescent tracers: 1) The hardened pieces of gel are easy to handle. 2) Applied to the spinal grey, the gel functions as a reservoir from which the FB diffuses freely into the surrounding tissue, preferentially into the more loosely arranged structure of the grey matter. No pressure is built up, as happens during injection, so that no tracer is squeezed into the surrounding funiculi. 3) There is no backflow of tracer through the needle track. 4) Damage to passing fibers due to imperfections of the injection technique or unwanted movements of the animal under anaesthesia, is minimal.

Thus, the presence of the two tracers in brain stem neurons means: 1) Any FB-containing cell in the brain stem projects at least to the cervical intumescence, but may reach other spinal levels as well; 2) Any NY-containing cell in the brainstem projects to or beyond the level of the NY gel implantation, but may also innervate more proximal levels of the cord, except for the cervical intumescence; 3) Any neuron containing both FB and NY (double-labeled neuron, see Fig. 3) projects to the cervical intumescence, as well as to or beyond the level of the NY gel implantation, but may also project to other spinal levels.

Experimental animals

In this study 40 lizards were used, varying in weight from 230 to 1130 g, with a total length of 44 to 72 cm, and a snout-vent length of 22 to 37 cm. The animals were housed in an air-conditioned room with a fixed temperature of 28 °C, and a natural dark-light cycle.

Surgical procedure

Prior to operation the animals received anaesthesia by inhalation of Ethrane, after which they were intubated; then, during operation a mixture of Ethrane, oxygen and nitrous oxide was administered. Under aseptic conditions a midcervical

(6^{th} spinal segment) laminectomy was performed, and after a longitudinal incision of the dura, two polyacrylamide-gels containing the fluorescent retrograde tracer "Fast Blue" (FB) were implanted unilaterally into the spinal grey matter, one caudal to the other, using a fine-tipped forceps.

After a survival time of 11 days, the animals were reoperated: under Ethrane anaesthesia a second laminectomy was performed, exposing either the lumbar (26^{th} and 27^{th} spinal segments) or the midthoracic (15^{th} and 16^{th} spinal segments) cord in the first (I) and second (II) experimental groups respectively. This time, five to seven polyacrylamide-gels containing the fluorescent retrograde tracer "Nuclear Yellow" (NY) were implanted ipsilaterally into all spinal funiculi. The animals survived another three days, which proved to be the optimum for "Nuclear Yellow", under the same conditions as before, and then they were perfused transcardially under deep Nembutal anaesthesia.

Survival time after FB application was not very critical, but had to be longer than 10 days for bright fluorescence. For NY, on the contrary, an optimum of three days was found; using longer survival times, the NY was leaking out of the labeled cells and staining the surrounding glial nuclei.

Histological procedure

Perfusion was performed in three steps with 1) physiological saline solution for 1 min., 2) 250 ml 4% paraformaldehyde in 0.1 M sodium phosphate buffer, pH 6.2, 3) a similar solution, pH 7.4. Ice-cold (4 °C) perfusion fluids were used, the animal being cooled in ice (0 °C). This rendered a brighter fluorescence then when perfusion took place at room-temperature. The brain and the spinal segments with the gel implants were removed, and post-fixed in the fixative of the last perfusion step to which 10% sucrose had been added, for 2-3 hours. Subsequently the brain was frozen in dry ice (CO_2), and cut on a freezing microtome into transverse sections of 30 μm, which were then collected in demineralized water and immediately mounted on glass slides coated with chromalum-gelatin (0.05 g chromalum and 0.5 g gelatin in 100 ml demineralized water). The experimental series, composed of one out of four sections from the caudal medulla oblongata up to the caudal diencephalon, was allowed to dry on the air at room temperature during 1 hour, and then coverslipped with DePeX (Gurr®) as mounting-medium. Stored in the dark at 4 °C the series remained in excellent condition for about 1 year. Adjacent sections were counterstained with cresylecht violet for topographical reference.

The spinal segments with the FB and NY gel implants were postfixed for 24 hours, and embedded in a 30% sucrose phosphate buffered solution (pH 7.4) containing 15% gelatin. This was stored overnight at room temperature in a 4% formaldehyde solution, rinsed in 30% sucrose phosphate buffer during 2 hours, and subsequently frozen in dry ice and cut into transverse sections of 40 μm. These were mounted as mentioned above.

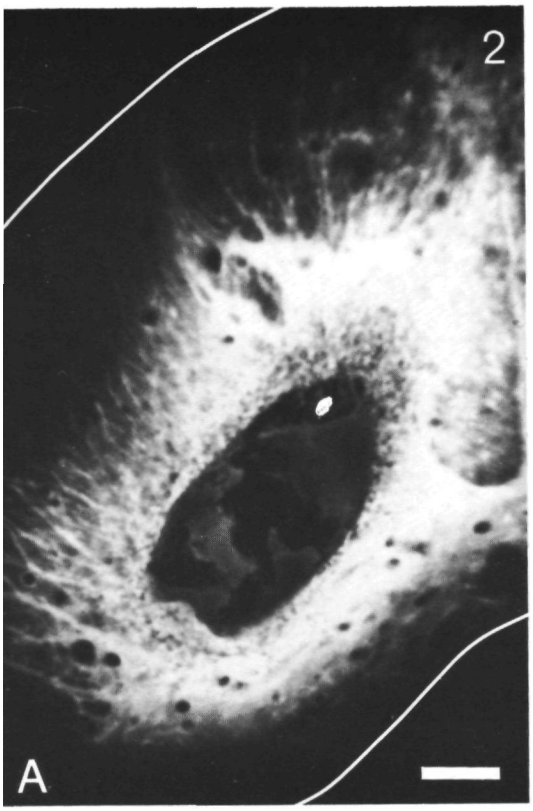
Preparation of the dye-containing gels

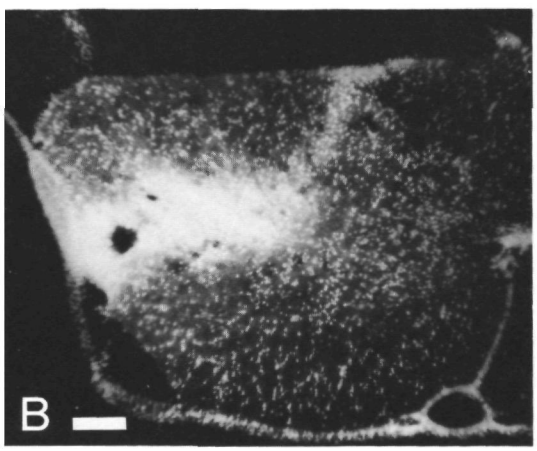
The preparation of gels according to Griffin et al. (1979) was slightly modified: to 16.75 μl of an acrylamide-bis solution (composed of 75% acrylamide (Serva®) and 15% n,n'-methylene-bis-acrylamide w/v (Serva®)) the following substances were subsequently added and mixed well: 5 μl dimethylsulfoxide (DMSO) (5%), 25 μl 0.1 M Tris-buffer pH 7.4 , 1 μl Temed (Bio.Rad®), and 2.5 mg of the dye "Fast Blue" (Dr. Illing K.G., Gross Umstadt, FRG) or 4 mg "Nuclear Yellow" (Hoechst®). To the 47.75 μl solution thus obtained, 2.5 μl ammonium persulfate (6%) were added while sturring well, and immediately sucked into a silastic tube with the desired diameter (0.12 to 0.21 mm), where polymerisation occurs within 1 minute. Then squeezing the contents of the tube onto a glass plate produces a gelatinous ribbon, that should be cut into pieces of the desired length (0.6 to 0.9 mm) at this moment. After drying the gels during one day at room temperature, they have hardened enough to be used succesfully, although hardening will continue for several days. The gels should be stored in the dark at room temperature, and can be used during at least three months.

Evaluation of the results

The sections were viewed with a Zeiss standard fluorescence microscope, equipped with an UV excitation filter (combination 487702) providing excitation light at a wavelength of 365 nm. Plotting of the retrogradely labeled neurons was achieved with the aid of an X-Y plotter (Kipp Instruments), connected to resistance transducers on the microscope stage (see Figs. 4 and 5 as examples). Over the plotted sections, the Nissl-stained pictures of the adjacent sections were projected for reference. Cell counts were made within 23 brain stem nuclei (see table 1), distinguishing three types of labeled cells: 1) "Fast Blue" labeled cells (FB cells) in which the blue fluorescent dye is present throughout the cytoplasm, leaving an unstained spot where the nucleus is found; 2) "Nuclear Yellow" labeled cells (NY cells) in which the white-yellow fluorescent dye is mainly present in the nucleus, leaving the cytoplasm unstained (or very weakly stained); 3) double-labeled neurons (DL cells) which show a white-yellow nucleus surrounded by blue cytoplasm (Fig. 3).

As a result of administration of very high doses of NY (Illert et al. 1982), or as a stage in the process of NY leakage out of the labeled neuron (Ben-





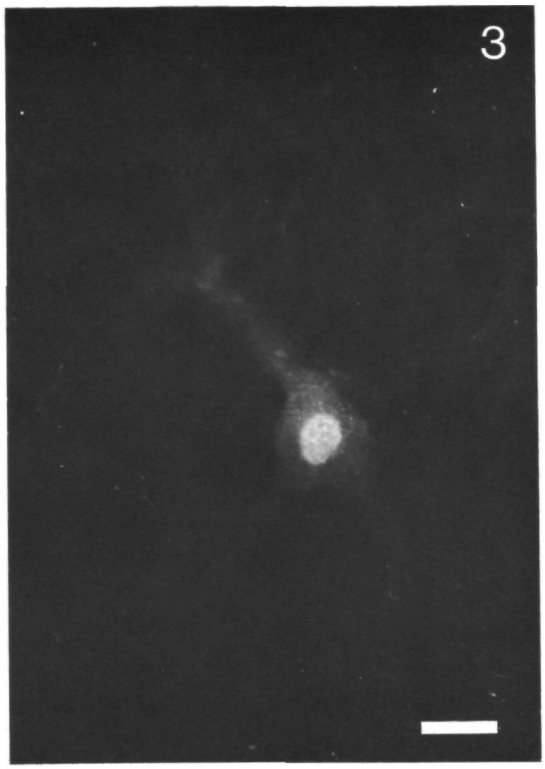


Fig. 3. A double-labeled neuron in the nucleus reticularis inferior (case 1), showing an intense "Nuclear Yellow" stained nucleus, surrounded by "Fast Blue" stained cytoplasm. (Bar: 30 μm)

Fig. 2. Photomicrographs of implantation sites. **A.** "Fast Blue" gel in the spinal grey matter of the cervical enlargement; note that the tracer is restricted to the direct environment of the gel. (Bar: 200 μm) **B.** One of the "Nuclear Yellow" gels in the lumbar enlargement of case 1; note the spread of the tracer throughout the spinal funiculi. (Bar: 200 μm)

tivoglio et al. 1980; Kuypers and Huisman 1984), not only the nucleus but also the cytoplasm may be stained, thus possibly masking a weak FB fluorescence in the same cell body. This phenomenon was encountered only incidentally in the present study, and could be overcome by switching to a different illumination filter in cases of doubt, viz., Zeiss 487703, providing violet excitation light at a wavelength of 405 nm, suppressing the NY fluorescence for a great deal, but not FB fluorescence.

The data thus obtained in seven lizards with lumbar, and five lizards with thoracic NY gel implantations were introduced in an APPLE II microcomputer, and evaluated numerically (see Appendix). Only those cases were selected for mathematical analysis in which the three conditions for a successful experiment were fulfilled: 1) cervical FB implantation was restricted to the unilateral spinal grey matter, and detailed histological observation could not reveal any damage to passing nerve fibers; 2) NY gel implants were restricted to the ipsilateral side; 3) the numbers of both FB and NY cells present in the brain stem were considerable.
In order to enhance comparison of the data from the different nuclei extending over different numbers of sections, the mean amounts of differently labeled cells per section were computed, for each of the 23 nuclei studied, in each animal. For comparison of the results obtained in the first series of experiments

(group I : lumbar NY gel implantations) with those of the second series (group II : thoracic NY gel implantations), the mean amounts of labeled cells per section in each experimental series were computed for each nucleus from the means found in the individual animals of the two groups. The standard errors (SE) to these values were obtained using the following formula:

$$SE = \frac{\sigma_{n-1}}{\sqrt{n}} = \frac{\sqrt{\Sigma\,(m-a)^2/(n-1)}}{\sqrt{n}}$$

in which σ_{n-1} is the standard deviation, a the mean number of labeled cells per section of a nucleus in one animal, m the mean amount of labeled cells per section in the experimental group I or II, computed from the means of the individual animals in the group concerned, and n the number of animals in the group.

Rather than cell counts of DL neurons in the different brain stem nuclei, their relative quantity reveals the degree of spinal collateralization of neurons within these nuclei. Therefore, the number of DL cells is also expressed as a percentage of the sum of the total amounts of FB and DL cells in each nucleus, using the following formula (from Peterson et al. 1975; Shinoda et al. 1977; Huisman et al. 1981, 1982):

$$\frac{\text{DL cells/nucleus}}{\text{FB cells/nucleus} + \text{DL cells/nucleus}} \times 100\%$$

The percentages of DL cells thus computed in individual animals for each of the 23 nuclei studied (see e.g. Fig. 11 for data from nucleus raphes inferior, nucleus reticularis inferior, nucleus vestibularis ventromedialis and nucleus ruber), were used to compute the mean percentages of DL cells ± SE in both experimental series, I and II, for each nucleus (see Figs. 5-7).

Results

Following a few notes on the aspect of the spinal gel implantation regions, the distribution of brain stem neurons in the monitor lizard, *Varanus exanthematicus*, labeled retrogradely from different levels of the spinal cord with the fluorescent tracers "Fast Blue" (FB) and "Nuclear Yellow" (NY), will be described. Attention will be paid to somatotopic aspects as well as to the individual characteristics of the projection patterns present in most of the 23 different nuclei studied (see table 1). The results of two parallel sets of experiments, i.e. with NY applied to lumbar and thoracic levels respectively, will be compared. The terminology used is mainly based on Butler and Northcutt (1973), Cruce and Nieuwenhuys (1974), ten Donkelaar and Nieuwenhuys (1979), and Newman and Cruce (1982). A few nuclei, however, have been delineated differently from the latter authors: the nucleus reticularis inferior (Ri) in *Varanus exanthematicus* corresponds to the nucleus reticularis inferior pars dorsalis (RID) of Newman and Cruce. These authors do not distinguish a nucleus reticularis inferior pars lateralis (Ril), which is found as a group of bilaterally labeled cells, lateral to the dorsal part of the nucleus reticularis inferior in *Varanus exanthematicus*. The nucleus reticularis superior pars lateralis (Rsl) in *Varanus exanthematicus* comprises the ventrolateral part of a similarly named nucleus (RSL) of Newman and Cruce, whereas the dorsomedial part of this nucleus is called nucleus reticularis superior (Rs) in *Varanus exanthematicus*.

Recently, an up-to-date atlas of the brain stem of the monitor lizard, *Varanus exanthematicus*, has been prepared (Wolters, 1985).

FB and NY gel implantation regions

In all cases, selected for mathematical analysis following the criteria mentioned before, the FB gels had invaded both the dorsal and ventral horn, but remained restricted to the spinal grey matter. From the differences in fluorescence intensity around the site of the FB gel implantation (Fig. 2 A) (see also Huisman et al. 1981, 1982; Kuypers and Huisman 1984) it may be concluded, that the maximum concentration of FB was present in the direct vicinity of the gel within a distance of 150 μm (golden-yellow fluorescence), generally restricted to the ipsilateral grey matter. This area, as viewed in transverse sections, is surrounded by a second 50-150 μm wide fluorescent zone of rapidly decreasing fluorescence intensity (from white to light blue) which contains large amounts of stained glial nuclei. This second area mostly involves parts of the funiculi directly adjacent to the grey matter, in which particularly fibers of propriospinal connections are present (Kusuma and ten Donkelaar 1980), as well as the medial part of the contralateral area X, as distinguished in various reptiles by Kusuma et al. (1979). Beyond this second zone the tissue shows a fairly high background fluorescence decreasing to dark blackblue at the edge of the section. In rostrocaudal direction there was a considerable spread of FB in the grey matter over a distance of 0.5 to 1 mm, thus ensuring a substantial uptake of the tracer by numerous nerve terminals at cervical level.

The gels containing NY were placed deliberately into the ipsilateral spinal funiculi at lumbar (I) or thoracic (II) spinal levels, thereby lesioning as many passing fibers as possible, to enhance uptake of the

dye. Following the tissue damage that was achieved repeatedly, it was sometimes difficult to obtain a proper reconstruction of the implantation site. However, if could be established that the multiple NY gel implants were restricted to the ipsilateral side, the experiments were used in our numerical analysis. In spite of our efforts to damage fibers in all spinal funiculi, only in the cases 9, 10 and 11 NY gels were found in the ventromedial part of the ventral funiculus. In the cases 3, 5, 7, 8, 10 and 12 only parts of the ventral part of the lateral funiculus were damaged by NY gels. In all cases, however, NY had spread extensively throughout all spinal funiculi, in addition to the high concentration of the dye in the spinal grey over a distance of 1.5 to 2 cm. In case 6 relatively little NY has been transported retrogradely, due to a spinal hemorrhage just rostral to the implantation site. The amounts of NY and DL cells are therefore relatively low, and in some nuclei the percentage of DL neurons is extremely low. These values have been disregarded in the numerical evaluation.

Distribution of FB and NY labeled brain stem neurons

Following FB gel implantation into the cervical intumescence and NY gel application to the lumbar enlargement (I) or thoracic levels (II) of the spinal cord, both FB and NY single-labeled neurons were found in many nuclei throughout the brain stem and caudal hypothalamus (see Figs. 4 and 5), e.g. the area lateralis hypothalami, the nucleus of the flm (Fig. 6), the nucleus ruber (Fig. 9), the locus coeruleus (Fig. 8) and subcoeruleus, the nucleus cerebellaris medialis, the vestibular nuclear complex (Fig. 7), the nucleus descendens nervi trigemini, the nucleus tractus solitarii, and many parts and subnuclei of the reticular formation (Fig. 10). All these nuclei studied thus project to the cervical enlargement as well as to thoracic or more caudal levels of the spinal cord.

The numbers of labeled cells vary in the different nuclei (see e.g. Figs. 4 and 5): numerous FB and NY cells were present e.g. in the contralateral nucleus ruber (Fig. 9), the ipsilateral locus coeruleus (Fig. 8), the ipsilateral nucleus vestibularis ventrolateralis and the nuclei reticulares inferior (Fig. 10) and medius; only a few labeled cells were found e.g. in the nucleus periventricularis hypothalami, the locus subcoeruleus, the nuclei vestibulares ventromedialis (Fig. 7) and descendens and parts of the reticular formation.

In general, the total amounts of NY and FB cells in the brain stem, as counted in the first series of experiments, tend to be equal (0 to 4.8 cells per nucleus per section, see table 1). However, the administered quantity of FB must be estimated significantly smaller then that of NY, because FB uptake takes place only via terminals of descending supraspinal fibers, present at the 6th cervical spinal

Abbreviations used in the figures

Alh	area lateralis hypothalami
Amb	nucleus ambiguus
Cerm	nucleus cerebellaris medialis
Coa	nucleus cochlearis angularis
cp	commissura posterior
DL	(number of) FB-NY double-labeled cell(s)
FB	(number of) "Fast Blue" single-labeled cell(s)
Fl	nucleus funiculi lateralis
flm	fasciculus longitudinalis medialis
Fun	nucleus funiculi dorsalis
Gp	nucleus geniculatus pretectalis
Iflm	nucleus interstitialis of the flm
Ipv	nucleus interpeduncularis, pars ventralis
Lc	locus coeruleus
Ll	nucleus lemnisci lateralis
Nflm	nucleus of the flm
nVIII	nervus vestibulocochlearis
NY	(number of) "Nuclear Yellow" single-labeled cell(s)
Ols	oliva superior
Opt	nucleus opticus tegmenti (= nucleus of the basal optic root)
Pb	parabrachial region
Ph	nucleus periventricularis hypothalami
Phg	nucleus prepositus hypoglossi
Prmr	nucleus profundus mesencephali, pars rostralis
Rai	nucleus raphes inferior
Ras	nucleus raphes superior
Ri	nucleus reticularis inferior
Ril	nucleus reticularis inferior pars lateralis
Riv	nucleus reticularis inferior pars ventralis
Rm	nucleus reticularis medius
Rml	nucleus reticularis medius pars lateralis
Rot	nucleus rotundus
Rs	nucleus reticularis superior
Rsl	nucleus reticularis superior, pars lateralis
Rub	nucleus ruber
Sol	nucleus tractus solitarii
tm	tectum mesencephali
Torc	nucleus centralis, torus semicircularis
Torl	nucleus laminaris, torus semicircularis
Vds	nucleus descendens nervi trigemini
Veds	nucleus vestibularis descendens
Vetg	nucleus vestibularis tangentialis
Vevl	nucleus vestibularis ventrolateralis
Vevm	nucleus vestibularis ventromedialis
Vh	nucleus ventralis hypothalami
VI	nucleus nervi abducentis
VII	nucleus motorius nervi facialis
XII	nucleus nervi hypoglossi
X	nucleus motorius dorsalis nervi vagi
%	percentage of FB-NY double-labeled cells

case 1

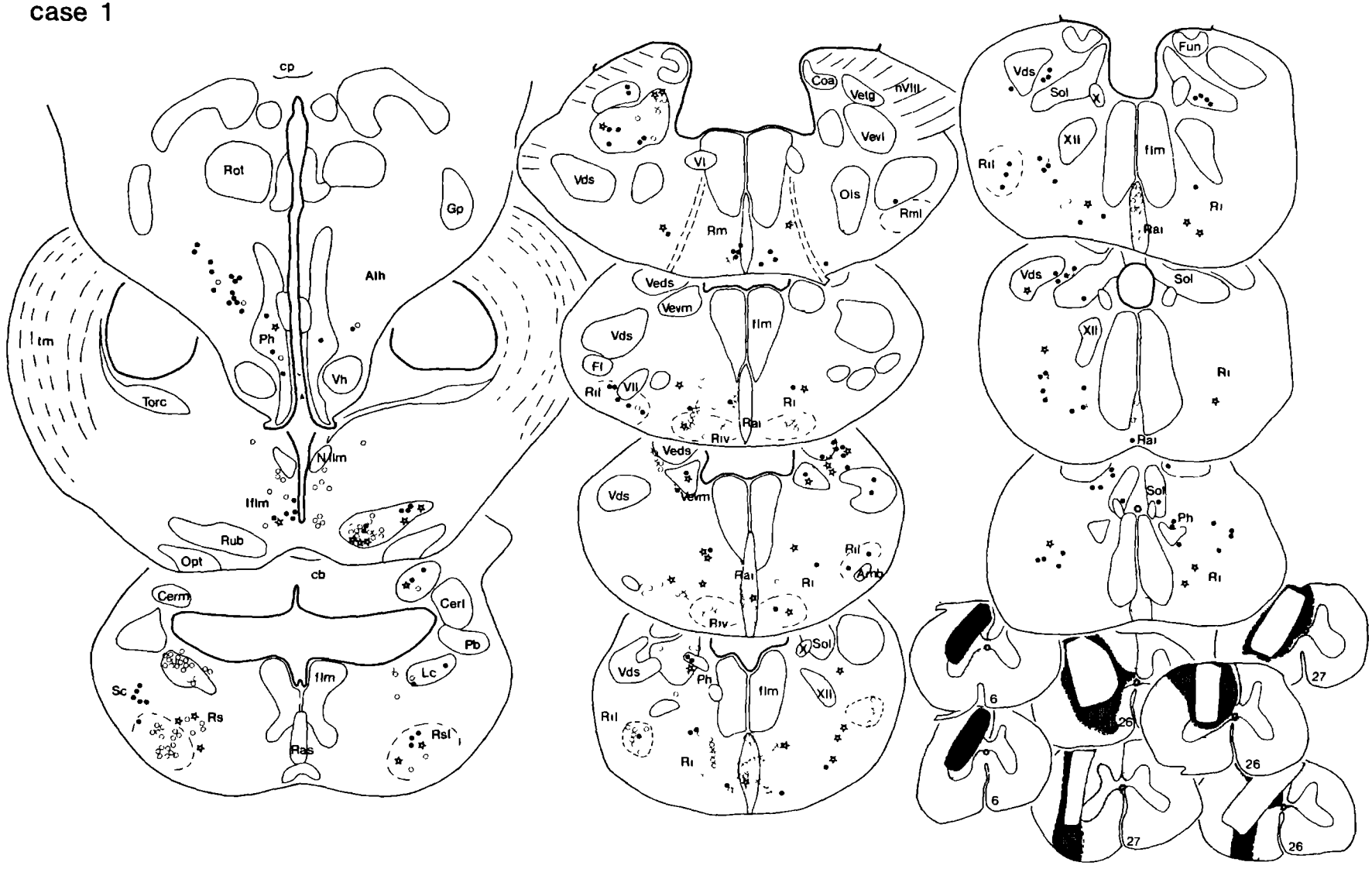


Fig. 4. The distribution of retrogradely labeled neurons in the brain stem of *Varanus exanthematicus* after "Fast Blue" (FB) gel implantations in the 6th spinal segment (i.e. the cervical enlargement) and "Nuclear Yellow" (NY) gel implantations in the 26th and 27th spinal segment (i.e. the lumbar enlargement). Single FB labeled cells are represented by dots, single NY labeled cells by open circles, and double FB-NY labeled neurons by asterisks. Numbers in the spinal cord sections indicate the level of the FB and NY gel implantations.

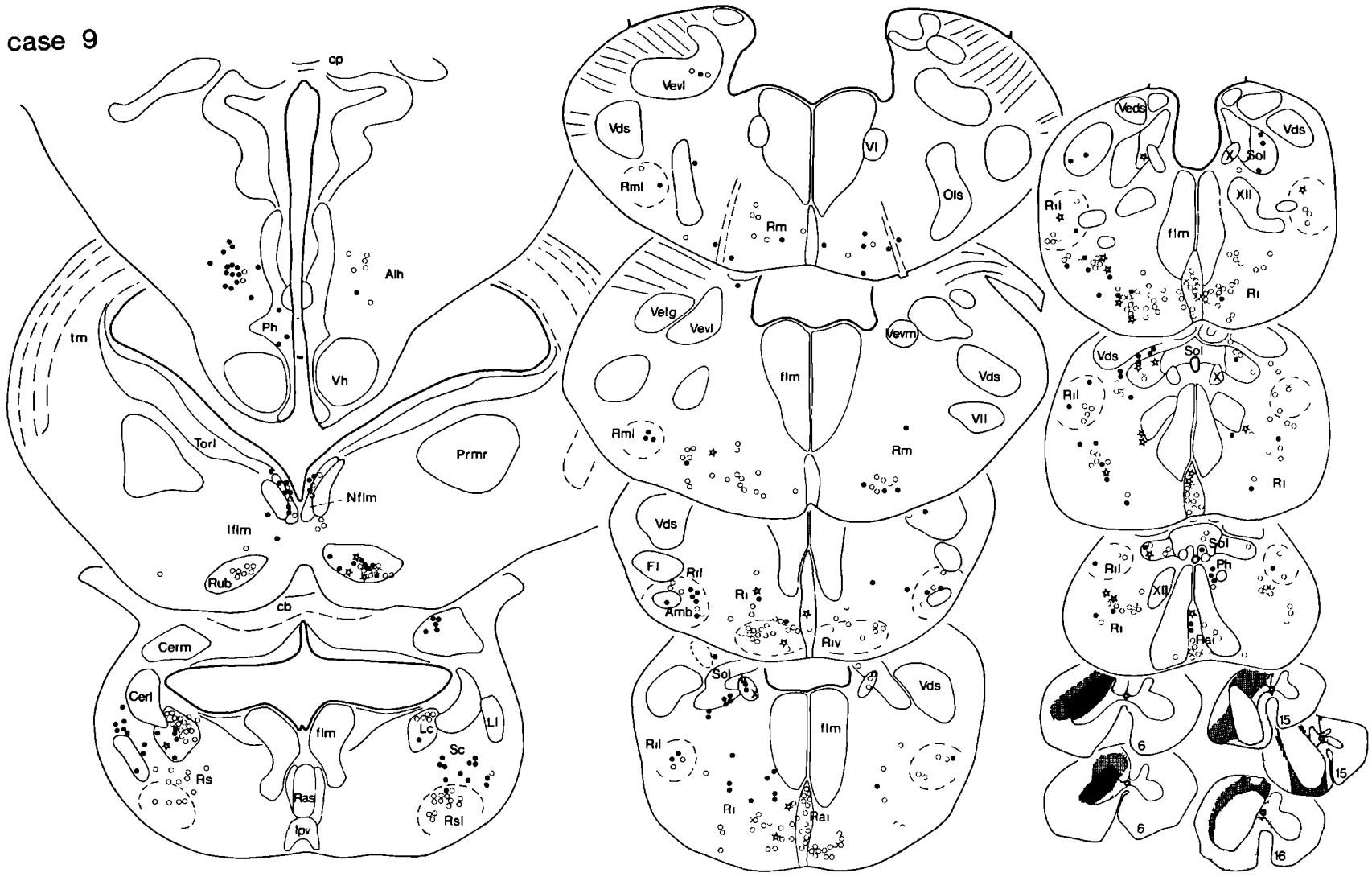


Fig. 5. The distribution of retrogradely labeled neurons in the brain stem of *Varanus exanthematicus* after "Fast Blue" (FB) gel implantations in the 6th spinal segment (i.e. the cervical enlargement) and "Nuclear Yellow" (NY) gel implantations in the 15th and 16th spinal segments (i.e. the midthoracic level). Single FB labeled cells are represented by dots, single NY labeled cells by open circles, and double FB-NY labeled neurons by asterisks. Numbers in the spinal cord sections indicate the level of the FB and NY gel implantations.

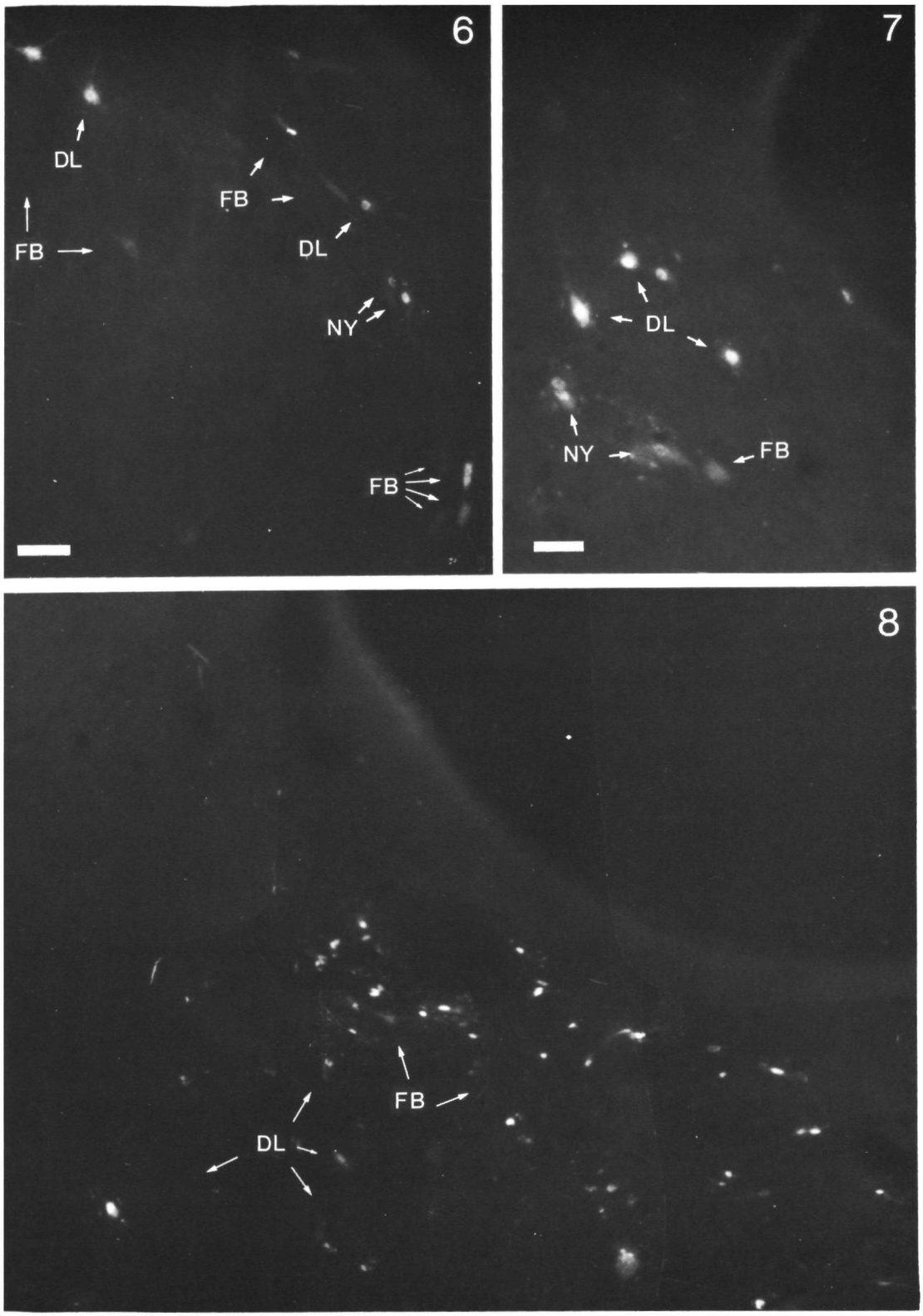
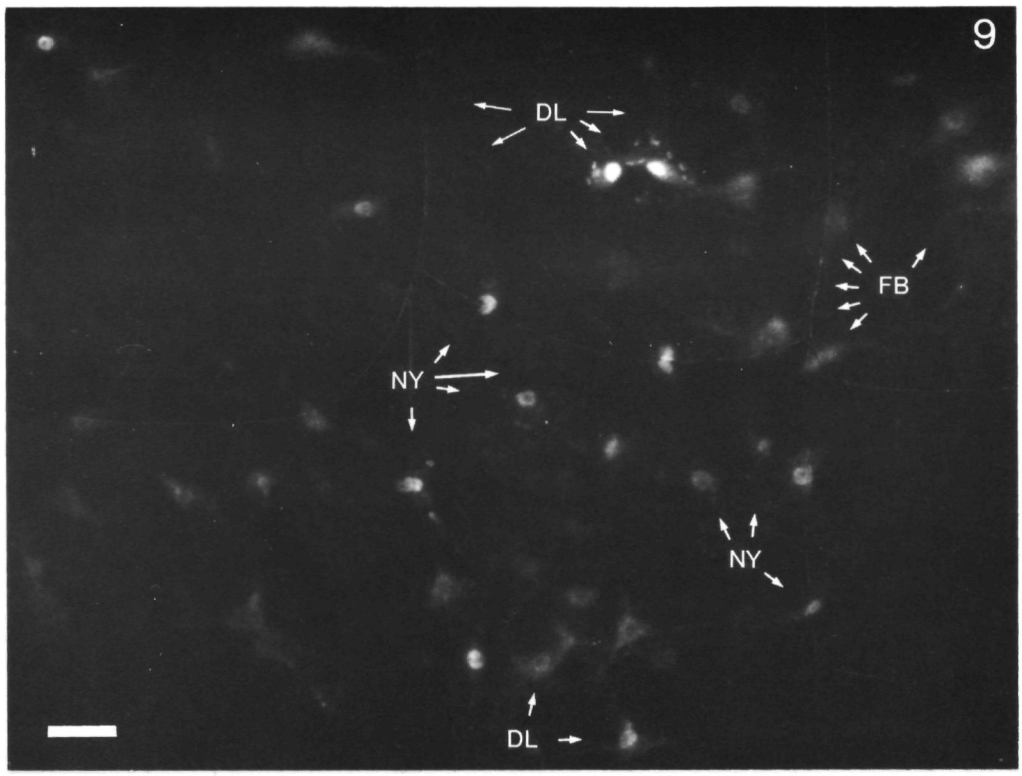


Fig. 6. Photomicrograph of several FB and NY single-labeled neurons and two FB-NY double-labeled neurons in the ipsilateral nucleus of the flm and the interstital nucleus of the flm in case 11. (Bar: 50 μm). **Fig. 7.** Photomicrograph of labeled neurons in the ipsilateral nucleus vestibularis ventromedialis in case 12. (Bar: 50 μm). **Fig. 8.** Photomontage of the ipsilateral locus coeruleus in case 12; note the high number of NY single-labeled neurons, whereas only two FB single-labeled and four FB-NY double-labeled cells are present in this section. (Bar: 100 μm)



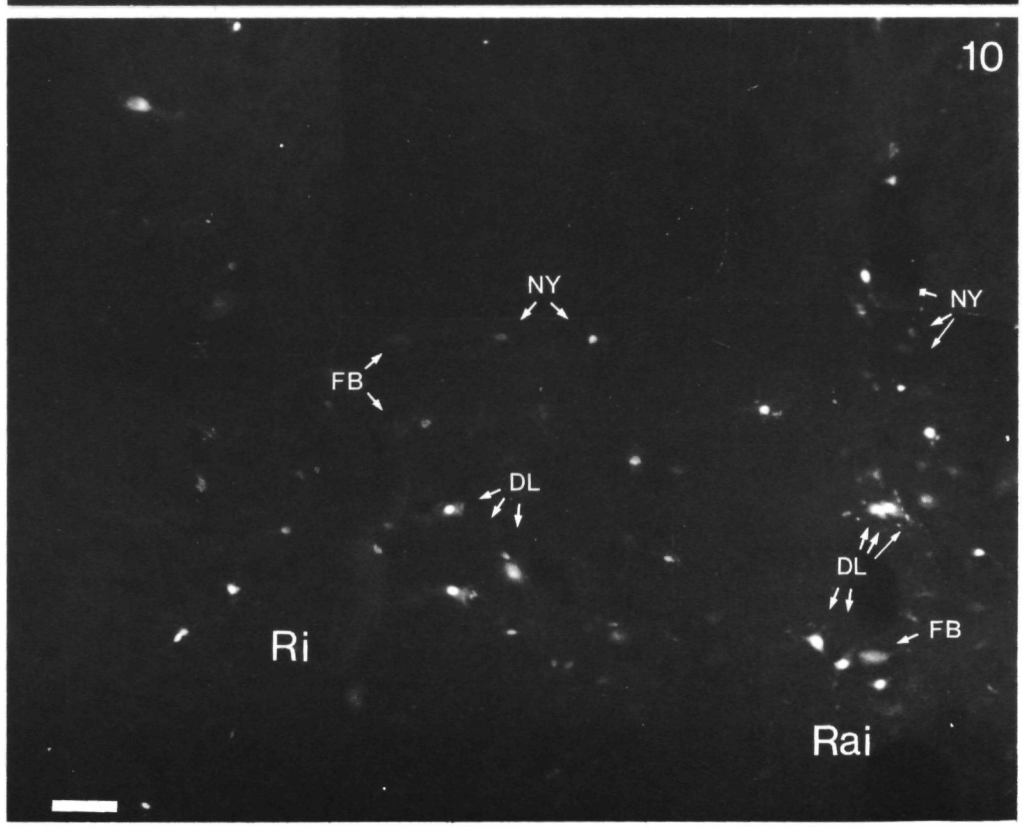


Fig. 9. Photomontage of the contralateral nucleus ruber in case 11, showing a conspicuously high amount of FB single-labeled neurons, as well as several NY single-labeled and FB-NY double-labeled neurons. (Bar: 50 um). **Fig. 10.** Photomontage of the nucleus raphes inferior and the ipsilateral nucleus reticularis inferior in case 12, at a level just rostral to the obex; note the particularly high number of NY single-labeled neurons, and the presence of several double-labeled neurons, whereas only few FB single-labeled cells are found. (Bar: 100 μm)

	FB			NY			DL			%DL		
	I		II	I		II	I		II	I		II
Rai	1 7		1 5	<<	**5.9**	<	**7.9**	0 5	<	1 0	**25.9**	<	**41 9**
Ri *(ipsi)*	**6.9**		**5.7**	<<	**11.4**		**13.9**	**1.8**		**2 8**	20 0	<	34 5
(contra)	4 1		4 1	<<	**6.5**		**7.9**	0 8		1 4	14 9		24 5
Ril	1 5		1 8		0 7	<	3 7	0 1	<	0 3	2 5	<	14 6
	1 8		1 7		0 6	<	2 9	0 0	<	0 2	3 6	<	9 8
Riv	0 9		1 6	<<	**6.5**		**6.7**	0 7		**1.5**	**47.6**		**45.1**
	0 8	<	2 2	<<	3 7		3 6	0 4	<	**1.5**	**31.0**	<	**40.7**
Sol	3 3		2 7	>>	0 7		1 3	0 1	<	0 4	4 0	<	12 3
	2 6		1 9	>	0 5	<	2 2	0 1	<	0 2	3 1	<	12 5
Veds	1 1		1 1		0 7		1 3	0 4		0 4	21 5		31 6
	1 4		2 3		1 6		1 4	0 5		0 9	**25.8**	<	**36.4**
Vds	1 6		0 5	>>	0 1		0 1	0 0	<	0 1	1 9		7 4
	0 1		0 2		0 1		0 1	0 0		0 0	6 3		5 6
Phg	0 1	<	0 3		0 1	<	0 3	0 0	<	0 1	3 3	<	33 3
	0 3		0 3		0 2		0 2	0 0	<	0 2	7 2	<	27 7
Vevm	1 3		1 2		0 6		0 7	0 2	<	0 7	12 9	<	33 7
	1 1		1 4		1 9		2 0	0 5		0 8	**29.0**		35 0
Vevl	**6.2**		**6.2**	>	2 0	<	5 3	0 6	<	**2.4**	10 2	<	29 0
	1 1	<	**4.8**		0 5	<	2 5	0 0	<	**2.4**	2 4	<	29 2
Rm	**5.1**		2 9	<	**6.6**		**9.7**	**1.1**		1 1	22 2		30 0
	3 7		2 8	<	3 8		5 7	0 4		0 6	12 0		19 9
Rml	2 3		2 5	>>	0 5	<	1 1	0 1	<	0 4	2 7	<	11 9
	1 0		1 1		0 8	<	2 0	0 3		0 3	16 5		15 6
Vpr	0 6	<	1 7		0 6	<	2 0	0 1	<	0 6	9 0	<	28 4
	1 6		0 6		0 6		1 1	0 5		0 6	**35.2**	<	**50.0**
Rs	2 5		2 3	<	4 7		6 5	0 8		0 6	**28.3**		27 7
	1 2		1 7		2 0		2 5	0 5		0 6	**23 6**		24 2
Rsl	0 9		1 4	<<	2 1		2 6	0 3		0 5	20 7		**40.4**
	1 4		1 8	<<	**6.8**		5 8	**1.0**		0 9	**29.0**		34 8
Cerm	0 5		0 8		0 0	<	0 4	0 0		0 0	0 0		0 0
	4 0		4 0	>	0 4	<	3 1	0 1	<	0 7	3 1	<	21 7
Lc	3 4		3 3	<<	**8.3**		**12.5**	0 4		0 7	9 8		17 8
	1 6		1 1	<<	2 4		2 1	0 2		0 2	6 1		11 3
Sc	2 9		2 9	>>	0 7		0 8	0 3	<	0 9	6 3	<	27 0
	1 2		1 1	>>	0 2		0 5	0 1		0 2	6 1		5 9
Rub	0 4		0 2	<<	1 7		3 3	0 0	<	0 2	6 7	<	30 0
	11.8		**9.6**	>	**5.0**	<	**8 0**	**2.2**	<	**3.2**	15 8	<	25 6
Nflm	3 8		4 0	>>	0 7		1 2	0 1		0 1	1 1		2 8
	1 8		2 6	>>	0 7		1 6	0 0	<	0 1	0 5	<	4 3
Iflm	4 3		4 2	>	0 9	<	3 2	0 4		0 9	6 9	<	21 2
	0 7		0 8		0 2		0 5	0 0		0 2	1 2		8 3
Ph	2 0		1 4		1 1		4 1	0 1	<	0 7	2 2	<	**42.2**
	0 3		1 1		0 9		0 2	0 0		0 0	0 0		0 0
Alh	**9.2**		**8.9**	>>	2 3		3 6	0 3		0 5	2 6		4 8
	0 7		1 7		1 4		1 8	0 0		0 0	0 0		4 8
MEAN	2 4		2 4		2 2		3 3	0 4		0 7	12 0		22 6
	±		±		±		±	±		±	±		±
SD	2 4		2 0		2 6		3 2	0 4		0 7	11 4		13 4

Table 1. Mean numbers of FB, NY and DL neurons per section, as counted in each of the 24 nuclei studied, and mean percentages of DL cells per nucleus Emphasized printing indicates higher values than mean + standard deviation (as indicated at the bottom of the table), calculated from the data in all nuclei studied Data from the first and second series of experiments (I and II) are placed in horizontal juxtaposition for easy comparison, between these two columns, < signs indicate for which nuclei the apparent difference between I and II exceeds means ± standard errors Ipsi- and contralateral counts are placed in vertical juxtaposition Between the columns of FB and NY cell counts a single or double < or > indicates by which tracer most neurons have been labeled retrogradely in the first (for FB) or second (for NY), or in both experimental series respectively In between the two columns that indicate the percentages of DL neurons, the < marks those nuclei in which, in the second experimental series, %DL was conspicuously raised The < and > signs have only been used when differences were greater than would be expected from the standard errors, calculated in the tested population For legibility these standard errors have been omitted in the table

segment, whereas NY is taken up via terminals present at two consecutive spinal segments, and moreover by all damaged descending fibers, passing through the levels of NY gel application. Since both tracers are equally sensitive (Kuypers and Huisman 1984), the lack of quantitative difference between the numbers of FB and NY cells can at least partly be explained by the larger distance over which the NY has to be transported (± 30 cm for NY and ± 10 cm for FB). This assumption is stressed by the counts made in the second series of experiments, where the total amount of NY cells (0.1 to 6.5 cells per nucleus per section, see table 1; transport distance for the NY ± 20 cm) was higher then that of FB.

The numbers of labeled cells per section in the different nuclei studied show a considerable variation. This is not only due to differences in the extent of the spinal projections arising from these nuclei, but also to the size of the nuclei in transverse sections, and to the cell densities in the various nuclei. This is particularly important with respect to very large and very small nuclei in the section, e.g. the nucleus reticularis inferior (± 6 FB cells per section (c.p.s.), ± 13 NY c.p.s., see table 1) and the nucleus prepositus hypoglossi (± 0.2 FB and NY c.p.s.) respectively. Since, however, the dimensions of many nuclei are not so extremely different in the transversal plane, mean numbers of cells can mostly be compared, and give an indication of the importance of a certain projection.

Most FB cells were found in the contralateral nucleus ruber (± 12 c.p.s., Fig. 9) and the ipsilateral area lateralis hypothalami (± 9 c.p.s.) (see table 1). Many FB cells were also present in the nucleus reticularis inferior (Fig. 10), the nucleus vestibularis ventrolateralis, especially ipsilaterally, the ipsilateral nucleus reticularis medius, the contralateral nucleus cerebellaris medialis and the ipsilateral nucleus interstitialis of the fasciculus longitudinalis medialis (flm). A few FB cells were found in the nucleus descendens nervi trigemini and the nucleus prepositus hypoglossi. Furthermore the ipsilateral nucleus cerebellaris medialis and nucleus ruber, known to give rise to a merely contralateral projection, and the contralateral nucleus interstitialis of the flm, projecting ipsilaterally, contained very few FB cells.

Both in the group I and group II experiments the highest amounts of NY cells were present in the nucleus reticularis inferior (especially ipsilaterally), the nucleus raphes inferior (Fig. 10), the ipsilateral nucleus reticularis inferior pars ventralis and nucleus reticularis medius, the ipsilateral locus coeruleus (Fig. 8), and the contralateral nucleus reticularis superior pars lateralis and nucleus ruber (table 1). Only a few NY cells were found in the nucleus descendens nervi trigemini, the nucleus prepositus hypoglossi, the ipsilateral nucleus vestibularis ventromedialis, the ipsilateral nucleus cerebellaris medialis, the locus subcoeruleus, the contralateral nucleus interstitialis of the flm and the contralateral nucleus periventricularis hypothalami.

In the following nuclei more FB than NY cells were present (table 1), suggesting a more significant projection to cervical than to lumbar or even thoracic spinal levels: the nucleus tractus solitarii, the nucleus descendens nervi trigemini, the ipsilateral nucleus reticularis medius pars lateralis, the locus subcoeruleus (Figs. 13 E-F), the nucleus of the flm and the ipsilateral area lateralis hypothalami (Fig. 14 F). In the nucleus vestibularis ventrolateralis (Fig. 14 B), the nucleus cerebellaris medialis (Fig. 14 C), the contralateral nucleus ruber (Fig. 14 D), and the nucleus interstitialis of the flm, the existing differences in the group I experiments fade in the second series of experiments.

Considerably higher amounts of NY cells than FB cells were found in the nucleus raphes inferior (Fig. 14 E), the nucleus reticularis inferior (Figs. 12 A-B), the nucleus reticularis pars ventralis (Figs. 12 E-F), the nucleus reticularis superior pars lateralis, especially contralaterally (Fig. 13 B), the ipsilateral locus coeruleus (Fig. 13 C), and the ipsilateral nucleus ruber.

Distribution of double-labeled neurons in the brain stem

In most of the 23 nuclei studied, double-labeled neurons (DL cells) were found, far less, however, than FB or NY cells (table 1, Figs.5-7). In general, more DL cells were present in the second series of experiments (II : NY applied to the thoracic cord) than in the first series (I : NY applied to the lumbar enlargement) (table 1).

Nuclei that showed conspicuous amounts of DL neurons in both experimental groups or in the second group only (i.e. the nucleus reticularis inferior pars ventralis and the nucleus vestibularis ventrolateralis) have been summarized in table 2, showing the mean numbers of DL cells per section in the individual cases. From these data the mean amounts of DL c.p.s. in each nucleus were computed for the entire series I and II of experiments (table 1).

In both series the highest density of DL cells was seen in the contralateral nucleus ruber (2.2 and 3.2 c.p.s. in groups I and II respectively). Furthermore, relative high amounts of DL cells were found in the nucleus reticularis inferior, especially ipsilaterally (1.8 and 2.8 c.p.s.), the ipsilateral nucleus reticularis medius (1.1 and 1.1 c.p.s.) and the contralateral nucleus reticularis superior pars lateralis (1.0 and 0.9 c.p.s.). Moreover, in the second group of experiments many DL cells were also found in the nucleus reticularis inferior pars ventralis (1.5 c.p.s.) and the nucleus vestibularis ventrolateralis (2.4 c.p.s.).

The degree of spinal collateralization of the descending supraspinal pathways can better be determined by means of the relative quantity of DL

Experimental group:		I						II					
Ri	*(i)*	0.8	0.8	1.1	0.4	4.0	1.1	3.9	3.3	1.9	1.0	4.5	3.5
Riv	*(i)*	0.2	0.4	0.8	0.3	0.3	0.0	2.2	1.3	1.1	(0.0)	3.3	0.3
	(c)	0.1	0.1	0.1	0.2	1.2	0.1	1.0	0.9	1.0	(0.0)	2.6	1.5
Vevl	*(i)*	0.0	1.3	0.0	0.0	1.2	0.0	2.0	(3.0)	3.0	(0.0)	2.1	2.0
	(c)	0.0	0.0	0.0	0.0	0.2	0.0	0.0	(0.0)	(0.0)	(0.0)	0.6	4.3
Rm	*(i)*	0.3	1.2	0.5	0.0	1.8	0.8	2.9	1.8	1.0	0.7	0.8	1.4
Rsl	*(c)*	0.0	0.4	0.6	0.1	0.9	0.0	4.1	0.3	0.8	1.3	1.3	1.4
Rub	*(c)*	0.7	1.6	3.0	0.6	3.8	1.6	4.2	2.2	2.9	2.6	4.5	3.7
Cases:		*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*

Table 2. Mean numbers of double-labeled neurons per section of the nuclei indicated, as counted in the individual cases; (i) = ipsilateral, (c) = contralateral. The values between brackets must be considered unreliable as a result of a very limited number of sections studied, and have not been used in further mathematical analysis.

cells than by absolute cell counts. Therefore the number of DL neurons is expressed as a percentage of the sum of FB and DL cells in a nucleus.

Percentages of DL cells (%DL) vary from 0% to ± 50% in the different brain stem nuclei studied, in both the first and second experimental groups (table 1). In the first series (NY applied to the lumbar enlargement) the mean was 12% ± 11.4%, in the second (NY applied to thoracic levels of the cord) the mean was considerably higher: 22.6% ± 13.4%. Highest percentages of DL cells (%DL) were found in the nucleus raphes inferior, the nucleus reticularis inferior pars ventralis, the contralateral nuclei vestibularis descendens and vestibularis ventromedialis, the contralateral nucleus princeps nervi trigemini, the nucleus reticularis superior pars lateralis, and (only in the second series of experiments) the ipsilateral nucleus periventricularis hypothalami. In the nucleus reticularis superior the percentages of DL cells (%DL) in the first and second groups of experiments were equally high, but only in the first group this percentage is relatively high. The lowest percentages of DL cells occur in the nucleus descendens nervi trigemini (especially in the second series of experiments), the ipsilateral nucleus cerebellaris medialis, the nucleus of the flm, the contralateral interstitial nucleus of the flm, the contralateral nucleus periventricularis hypothalami, and the area lateralis hypothalami (table 1).

Somatotopic organization

In none of the nuclei studied, including the nucleus ruber, the vestibular nuclear complex and the reticular formation, a clear-cut somatotopy was found, neither when viewed in the transversal plane nor in the sagittal plane: FB and NY cells were intermingled in an unpredictable way, and DL neurons were never restricted to particular parts of a nucleus (see Figs. 4, 5, 8-10). Two regions in the brain stem showed some sort of somatotopic organization, in which more than one nucleus is involved: 1) The caudal rhombencephalon (Figs. 4 G-J) . Here the majority of NY cells is present in its ventromedial half, whereas most FB cells are found in the dorsolateral part, i.e. the nucleus reticularis inferior pars lateralis. This "somatotopy", however, fades in the second group of experiments (Figs. 5 G-J). 2) The isthmic level (Figs. 4 C, 5 C), where the majority of FB cells is found in the area ventrolateral to the locus coeruleus: the locus subcoeruleus (see Wolters et al.'84). In the locus coeruleus proper (Fig. 8), and the nucleus reticularis superior pars lateralis much more NY than FB cells are present.

In neither of these two regions a preferential localization of DL cells in the transitional area between FB and NY cells, could be observed.

Comparison of the results from experimental series I and II

The amounts of FB cells were about equal in the first and second experimental series (table 1). This holds for the total amounts in the brain stem as well as for the numbers of FB cells in the individual nuclei studied. The apparent differences in most nuclei (table 1) remained within the confines of means ± standard errors. However, in 4 of 45 structures analysed (raphe, and 22 nuclei ipsi- and contralateral) the amount of FB cells was higher in group II than in group I experiments, viz., in the contralateral nucleus reticularis inferior pars ventralis and nucleus vestibularis ventrolateralis, and the ipsilateral nucleus prepositus hypoglossi and nucleus princeps nervi trigemini.

The total amount of NY cells in the brain stem was raised in the second series of experiments. Similarly, most nuclei studied individually showed an increase of NY cells in the second series (table 1). This increase exceeds the limits of means ± SE in 13 of 45 structures analysed, e.g. in the nucleus raphes inferior (Fig. 14 E), the nucleus reticularis inferior

pars lateralis (Fig 12 B), the nucleus vestibularis ventrolateralis (Fig 14 B), the nucleus reticularis medius pars lateralis, the nucleus cerebellaris medialis (Fig 14 C) and the contralateral nucleus ruber (Fig 14 D)

In most of the nuclei showing a higher number of NY cells in the group II experiments, a concomitant increase of DL cells was found In addition, more DL cells in group II than group I were found in some nuclei that did not show an obvious increase of NY cells (table 1), such as the contralateral nucleus reticularis inferior pars ventralis (Fig 5 F), and the ipsilateral nucleus vestibularis ventromedialis and locus subcoeruleus (Fig 13 E)

In about 50% of the nuclei studied the %DL was conspicuously raised in the series of experiments in which NY was applied to midthoracic levels of the spinal cord (group II) as compared to the group I experiments, those with the NY gel implantations in the lumbar enlargement (table 1), e g in the nucleus raphes inferior (Figs 11, 14 E), the ipsilateral nucleus reticularis inferior (Figs 11, 12 A), the nucleus reticularis inferior pars lateralis (Figs 12 C-D), the nucleus tractus solitarii, the nucleus vestibularis ventrolateralis (Fig 14 B), the nucleus princeps nervi trigemini, the contralateral nucleus cerebellaris medialis (Fig 14 C) and the nucleus ruber (Figs 11, 14 D)

In the rest of the nuclei studied, e g the nucleus descendens nervi trigemini, the nucleus reticularis medius, the nucleus reticularis superior and nucleus reticularis superior pars lateralis (Figs 13 A-B) and the locus coeruleus (Figs 13 C-D), the %DL remained about equal in the first and second experimental series

Discussion

In the present study the multiple retrograde fluorescent tracer technique was used to investigate collateralization of the descending pathways from the brain stem to the spinal cord in a lizard, *Varanus exanthematicus*. Single as well as double labeled

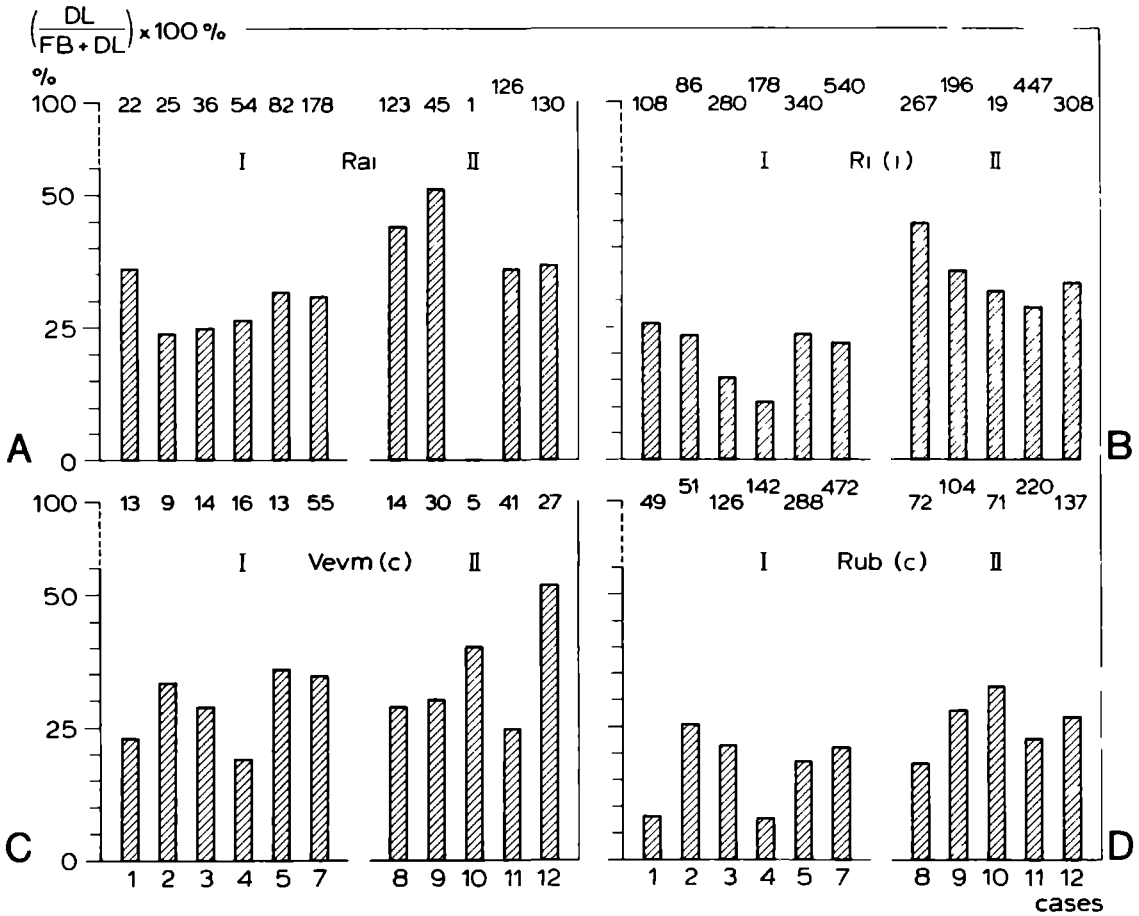


Fig. 11. Percentages of double FB-NY labeled cells in the individual experiments of group I (cases 1-5 and 7, NY gel implants in the lumbar enlargement) and group II (cases 8 12, NY gel implants in midthoracic spinal levels), as computed for the nucleus raphes inferior, the ipsilateral nucleus reticularis inferior, the contralateral nucleus vestibularis ventromedialis and the contralateral nucleus ruber Numbers at the top of the histograms represent the sum of the absolute FB and DL cell counts in each experiment, indicating the 100% level in the individual cases

neurons were found in most of the brain stem nuclei known to give rise to spinal projections in reptiles (ten Donkelaar et al. 1980; Cruce and Newman 1981; Wolters et al. 1982; Woodson and Künzle 1982; Newman et al. 1983; ten Donkelaar et al. 1983; ten Donkelaar and de Boer-van Huizen 1984a). Part of these observations confirm earlier HRP-data in *Varanus exanthematicus* (ten Donkelaar et al. 1980; Wolters et al. 1982; ten Donkelaar and de Boer-van Huizen 1984a), demonstrating, moreover, a much higher sensitivity of the fluorescent tracers as compared with HRP (see also ten Donkelaar and de Boer-van Huizen 1984b; Martin et al. 1981a,b), resulting in a larger number of labeled cells. This may explain why in the present study certain nuclei, previously considered to project only to cervical or high thoracic levels of the spinal cord, were found to have at least modest projections to the lumbar intumescence as well.

The presence of a few labeled cells in the ipsilateral parts of nuclei known to project merely contralaterally, e.g. the nucleus ruber, the nucleus reticularis superior pars lateralis and the nucleus cerebellaris medialis, may be explained in the first place by some unavoidable spread of tracer from the unilateral gel implantation site to the contralateral side. However, very modest ipsilateral projections from the mentioned nuclei, which remained unnoticed in degeneration or HRP experiments, cannot be excluded.

For numerical evaluation of the results, only 12 animals were used in accordance to the selection criteria mentioned before. Nevertheless, in this homogenous group of experiments important differences in cell counts were found in the individual animals; even the percentages of DL neurons showed considerable variation within each experimental series with NY implantations at the same spinal levels (see e.g. Fig. 11), a variability that was also found in mammals (Huisman et al. 1981 table I; Huisman et al. 1982 table IV). This made computing necessary for evaluation and comparison of the results obtained from the first and second experimental series.

The considerable differences in cell counts in the various brain stem nuclei have been associated with the extent of their spinal projections. Partly, however, these differences might be due to selective affinities of the tracers to the different descending systems (Kuypers and Huisman 1984). The use of longer survival times might alter the ratios found in the present study. Therefore, mutual comparison of the data from different nuclei should be interpreted with caution.

For critical tests in a statistical analysis, the number of successful experiments as well as the number of observations within each experiment would not have been sufficient in the present study. Nevertheless the numerical evaluation in a qualitative sense gives substantial evidence for certain trends in the population.

Spinal projections and collateralization of descending supraspinal pathways

The total amounts of FB and NY cells in the brain stem were nearly equal in the first group of experiments. In some nuclei, however, more FB cells were present than NY cells, or vice versa. This may indicate a preferential projection from certain nuclei to cervical or lumbar spinal levels, respectively.

In the first and second series of experiments, the number of FB cells remained fairly constant in most nuclei studied. The fluctuations herein (table 1; Figs. 12-14) may be due to several factors, such as the size and exact location of the FB gel, the transport distance (or the size of the animal), the physical condition of the animal, the brilliance of fluorescence after histological processing. The constant amount of FB cells, however, cannot easily be reconciled with the conspicuous increase of DL cells in the experimental group II. FB cells in group I may partly represent collateralizing neurons, projecting to e.g. lower thoracic levels, wich will turn into DL cells if NY is applied to the thoracic spinal cord. The nearly constant amount of FB cells in groups I and II could be explained if the increase of DL cells would be relatively small, but if %DL is considerably raised in group II, a decrease of the number of FB cells would be expected, as is seen in e.g. in the nucleus raphes inferior, the ipsilateral nucleus reticularis inferior and the nucleus ruber. Thus it appears that in the group II experiments FB has been taken up by more fibers terminating in the cervical intumescence than in the group I experiments, although all experimental conditions in both series were kept constant.

The amount of NY cells, however, was almost invariably raised in any of the nuclei studied in the second group of experiments. This can be explained by the presence, at thoracic level, of a greater number of descending supraspinal fibers in the spinal funiculi, damaged by implantation of the multiple NY gels. The "extra" fibers found here may in part be collaterals; in that case a concomitant

Figs. 12-14. Histograms showing mean absolute cell counts (hatched columns; values, varying from 0 to 15 cells per section: see left side Y-axis) and mean percentages of DL cells (black columns; values varying from 0 to 50%: see right side Y-axis) in some of the brain stem nuclei studied. Data from the first (I) and the second (II) experimental series are pictured in the same blocks in juxtaposition. The blocks at the left side of Figs. 12 and 13 show the data from the ipsilateral side, those on the right the data from the contralateral side of the nuclei. In Fig. 14 data from either the ipsilateral (i) or contralateral (c) side of some nuclei are pictured.

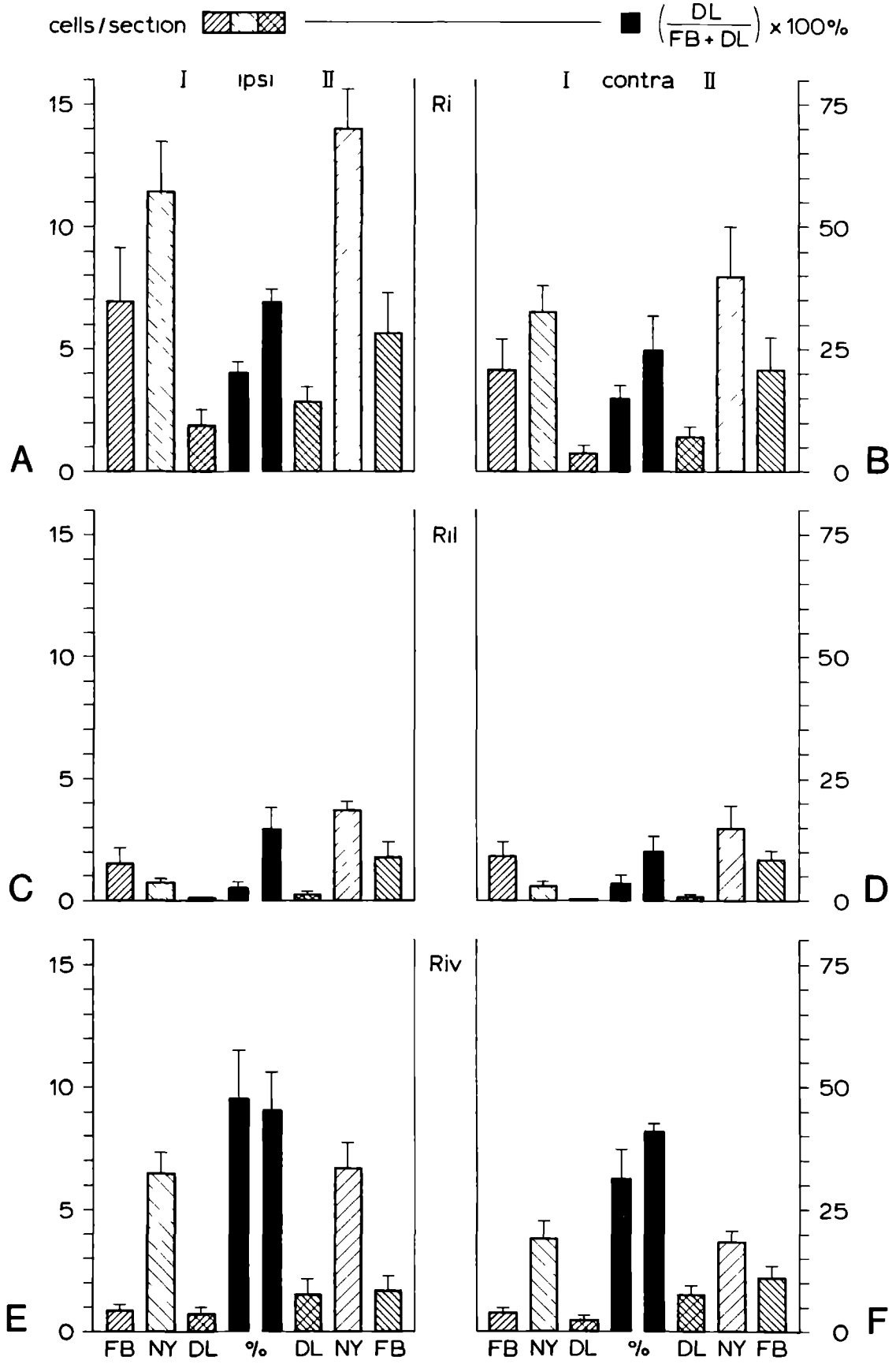

cells / section
(DL / FB + DL) x 100%
I ipsi II
I contra II
Ri
Rii
Riv
A
B
C
D
E
F
15
10
5
0
75
50
25
FB NY DL % DL NY FB

Fig. 12

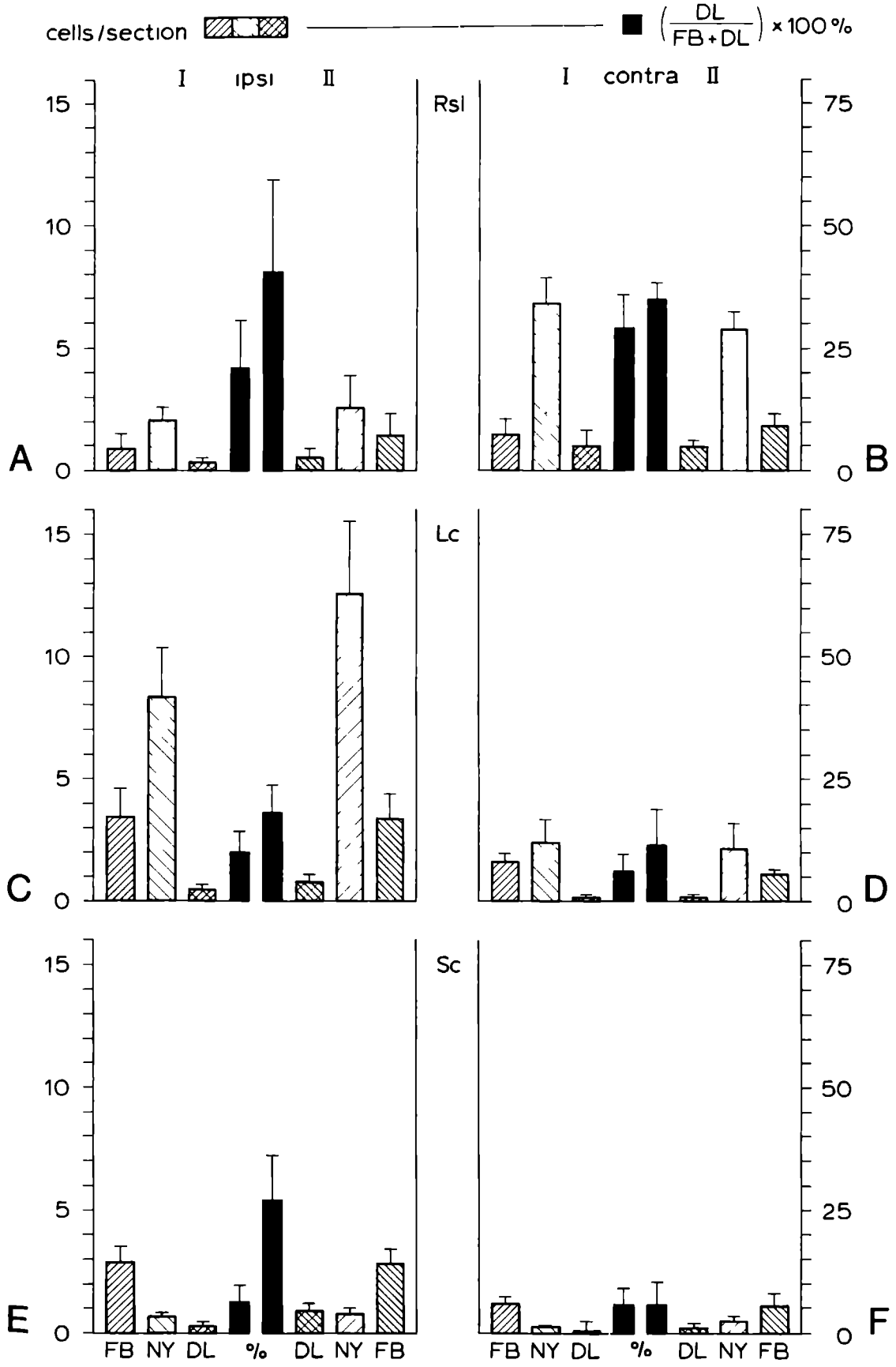

cells/section
$\left(\frac{DL}{FB+DL}\right) \times 100\%$
I ipsi II
I contra II
Rsl
Lc
Sc
A
B
C
D
E
F
15
10
5
0
75
50
25
FB NY DL % DL NY FB

Fig. 13

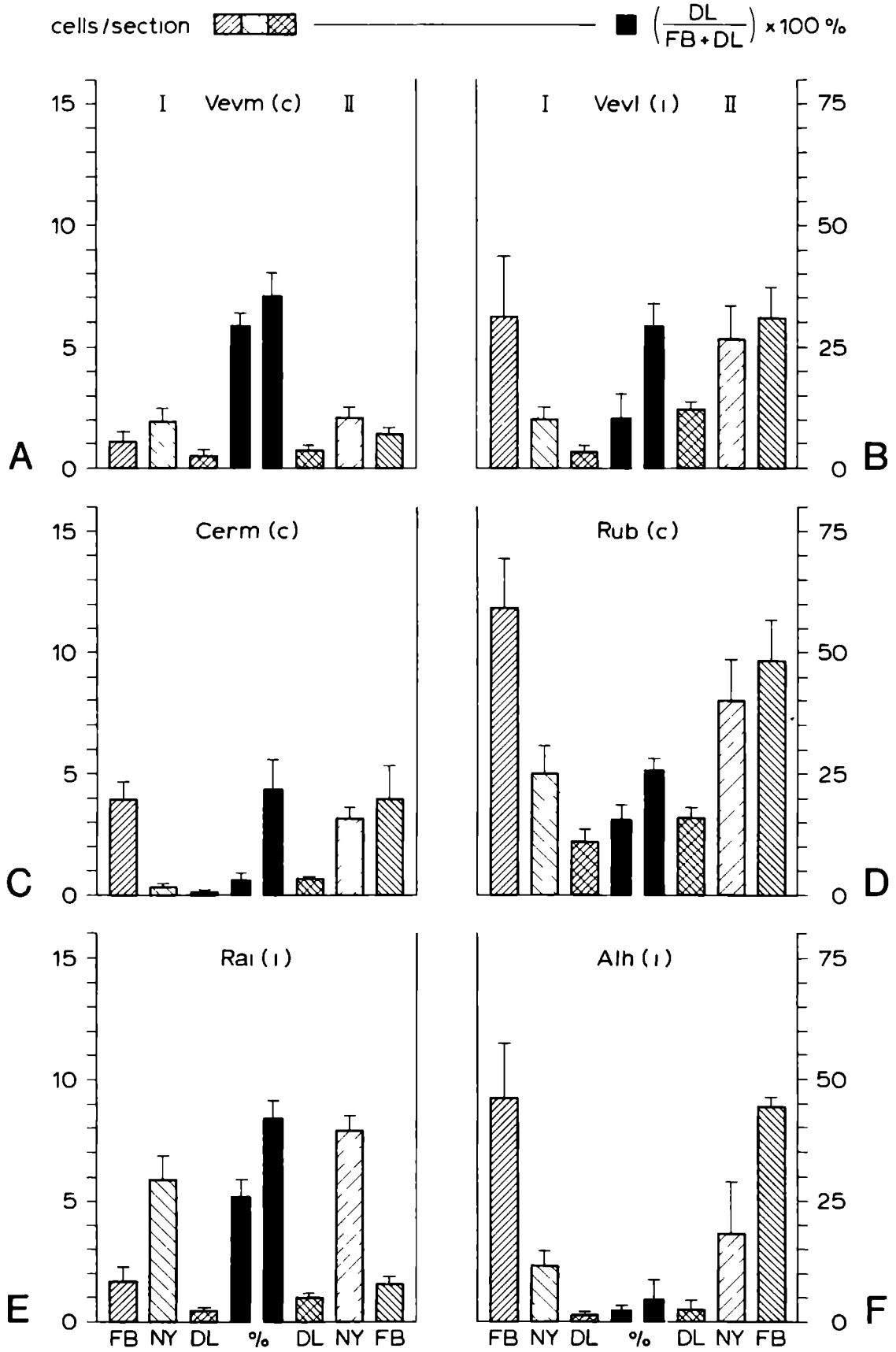

cells/section
$\left(\frac{DL}{FB+DL}\right) \times 100\ \%$
I Vevm (c) II
I Vevl (l) II
Cerm (c)
Rub (c)
Ral (l)
Alh (l)
A
B
C
D
E
F
15
10
5
0
75
50
25
FB NY DL % DL NY FB

Fig. 14

rise of DL cells is seen. Furthermore, merely the shorter transport distance of the dye in the group II experiments may contribute to the higher amount of NY and DL cells in group II.

As mentioned before, the relative quantity of DL neurons is more suitable for expressing the degree of collateralization than mere cell counting. For reference to other studies, the percentage of DL neurons (%DL) is expressed as the ratio of DL cells and the sum of DL and FB cells (Peterson et al. 1975; Shinoda et al. 1977; Huisman et al. 1981, 1982).

If, in a certain nucleus, %DL is low, i.e. few collaterals to lower spinal levels are given off by the neurons projecting to the cervical enlargement, this might indicate a more focussed spinal projection from such nucleus via non-branching fibers, whereas a high %DL suggests a rather global information to the spinal cord (Huisman et al. 1981, 1982). This, however, is not necessarily so while the former data also may fit in with highly collateralizing pathways that exclusively do not project to the cervical intumescence, yet supplying a rather global information to the spinal cord, and the latter data with sparsely branching pathways that innervate specific spinal cord regions, thus constituting more focussed projections.

The increase of %DL in group II as compared to group I, as seen in e.g. the nucleus raphes inferior, parts of the nucleus reticularis inferior, the nucleus tractus solitarii, the vestibular nuclear complex, the nucleus princeps nervi trigemini, the contralateral nucleus cerebellaris medialis and the nucleus ruber, indicates the presence of branching neurons, that project to the cervical enlargement as well as to or beyond the midthoracic level, terminating at various spinal levels, while the number of these fibers is decreasing more caudalward in the spinal cord (Fig. 15 A). A certain degree of specificity may thus be attributed to these collateralizing projections.

The increase of NY or DL cells and of %DL in the second experimental series found in most parts of the vestibular nuclear complex, the nucleus cerebellaris medialis and the nucleus interstitialis of the flm, which all project to the spinal cord via the ventral funiculus, might be explained by the fact, that only in the second series NY gels had really invaded this funiculus. In that case, however, the ipsilateral nucleus vestibularis descendens, nuclei reticulares medius and superior, and locus coeruleus, which project via the same funiculus, should show a similar increase of NY uptake as well. Since this is not the case, it may be assumed, that the former nuclei, in contrast to the latter, send collaterals to different levels of the spinal cord, decreasing in number more caudalward. If %DL remains constant in the first and second experimental series, as e.g. in the nucleus descendens nervi trigemini, the nucleus reticularis medius, all parts of the nucleus reticularis superior, and the locus coeruleus, we may conclude that all branching neurons, projecting from these nuclei to the cervical intumescence, that reach the midthoracic level of the spinal cord, continue their course, (while giving off new collaterals or not), to reach the lumbar intumescence or even lower spinal levels. Although specificity seems to be lacking in such a case, it must be emphasized that this labeling pattern also fits in with a highly focussed projection to both the cervical and lumbar enlargements only (Fig. 15 B).

In some nuclei, e.g. the contralateral nucleus reticularis inferior pars ventralis and the ipsilateral nucleus tractus solitarii, nucleus vestibularis ventromedialis, and locus subcoeruleus, the amount of DL cells is raised in the second experimental series, leaving the number of NY cells unchanged. This suggests that in these nuclei at least three neuron populations with a different projection pattern are present: 1) neurons that project to the cervical enlargement, that may give off collaterals to different spinal levels, but not beyond the midthoracic levels; 2) neurons that project to the lumbar enlargement or beyond, that may give off collaterals to other spinal levels, but not to the cervical enlargement; 3) collateralizing neurons that project to the cervical enlargement as well as to thoracic or lower levels of the spinal cord, showing a decrease of neurons reaching more caudal levels of the cord.

In the contralateral nucleus reticularis medius pars lateralis the amount of NY cells was raised in group II, leaving the number of DL cells constant. Here all collaterals from neurons projecting to the cervical enlargement that reach the midthoracic level, also reach the lumbar enlargement, whereas neurons that do not project to the cervical enlargement project to different levels of the spinal cord, decreasing in number when reaching more caudal levels.

Somatotopic aspects

The lack of a somatotopic organization of the descending pathways in *Varanus exanthematicus* was not entirely unexpected. Apart from HRP data, which show that certain brain stem nuclei in reptiles apparently do not project beyond cervical or high thoracic levels (ten Donkelaar et al. 1980; Woodson and Künzle 1982), so far no evidence for a clearcut somatotopy of the spinal projections in reptiles has been gathered. This is in keeping with data in mammals only as regards the nucleus raphes inferior and most parts of the reticular formation (Hayes and Rustioni 1981; Martin et al. 1981a; Huisman et al. 1981, 1982). However, a subtle topography of the reticulospinal projections was found in the opossum (Martin et al. 1981c) and the rat (Zemlan and Pfaff 1979; Satoh 1979). A somatotopic segregation in rostrocaudal and dorsoventral direction of reticulospinal neurons in cat was found by Peterson and co-workers (1975) for neurons projecting to

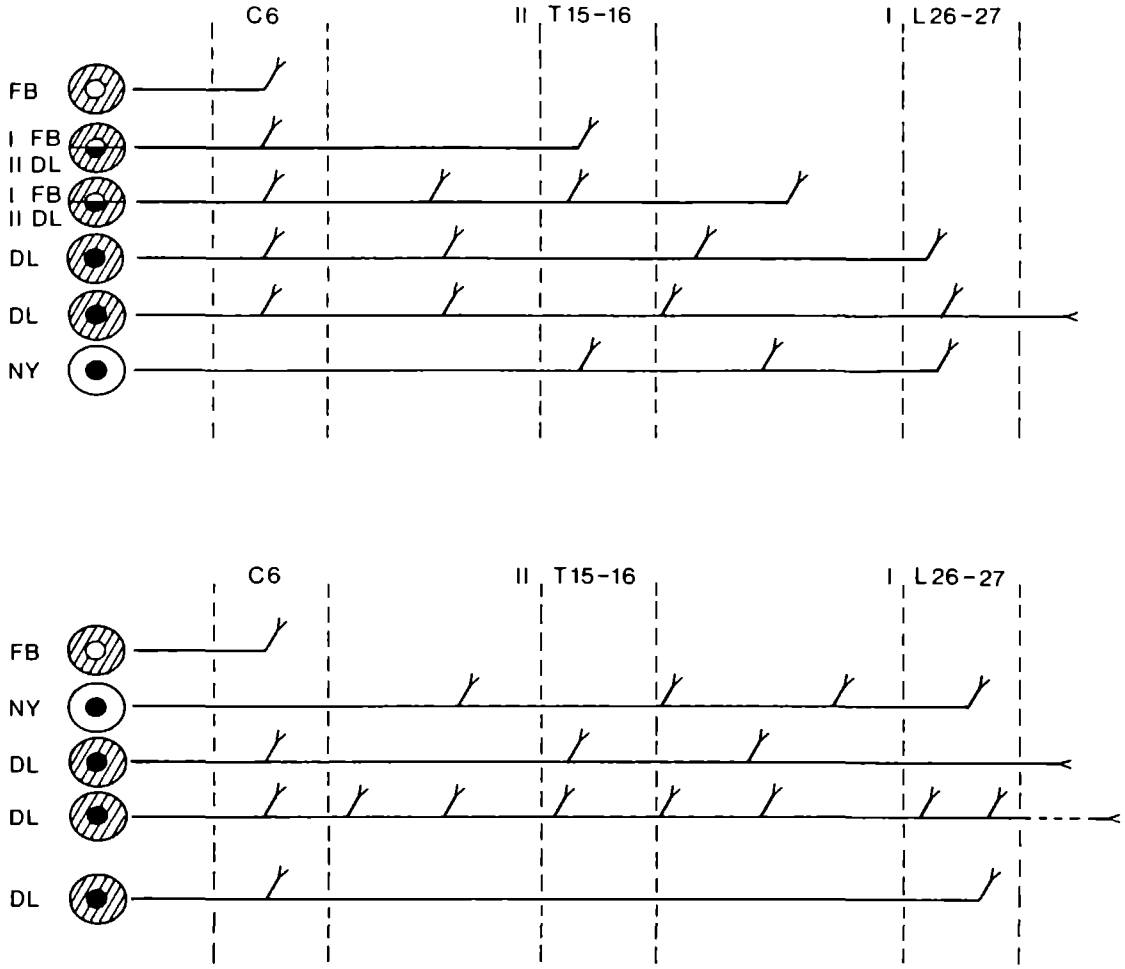


Fig. 15. Schematical representation of two different collateralization patterns which underly the observations that **A.** in certain nuclei, %DL increases if NY is applied to thoracic levels of the spinal cord, as compared to lumbar NY gel implantations; **B.** in other nuclei, %DL remains unaffected by the level of NY application. The latter pattern may suit long, frequently branching descending systems with global information to many parts of the spinal cord, but may also well fit in with highly selective descending pathways, e.g. exclusively focussing on both intumescences, influencing specifically limb motor activity.

spinal levels rostral to the 4th cervical segment and beyond, respectively, whereas no segregation of neurons projecting to the cervical or lumbar enlargement was found. The present study did not focus on these high cervical projections, rostral to the cervical enlargement.

The lack of a distinct rubrospinal somatotopy in *Varanus exanthematicus* so far only corresponds with data in the pigeon (Wild et al. 1979; Cabot et al. 1982) and in the opossum (Martin et al. 1981b), whereas in other mammalian species studied (e g. tree shrew, rat, cat, monkey) a somatotopic organization of the rubrospinal projection exists (Pompeiano and Brodal 1957a; Nyberg-Hansen and Brodal 1964; Miller and Strominger 1973; Murray et al. 1976; Shinoda et al. 1977; Kneisley et al. 1978; Castiglioni et al. 1978; Murray and Gurule 1979; Zemlan et al. 1979; Hayes and Rustioni 1981; Huisman et al. 1981, 1982; Padel et al.1981; Watkins et al. 1981). In opossum a segregation has been demonstrated for rubral neurons projecting to the lateral medulla oblongata, the inferior olive and the spinal cord, respectively, although a certain overlap exists (Martin et al. 1983). Comparably, rubral neurons in *Varanus exanthematicus* seem to be segregated as to their projection to the spinal cord (present study; Wolters et al. 1982) and to the lateral cerebellar nucleus (Bangma and ten Donkelaar 1982); the rubrospinal neurons are found throughout the red nucleus, the rubrocerebellar neurons presumably in the medial part only. It should be noted that in the rat nearly all

rubrocerebellar fibers represent collaterals of rubrospinal neurons, and that at least 37% of the rubrospinal neurons give rise to such cerebellar collaterals (Huisman et al. 1983).

A somatotopic organization of the vestibulospinal projection from the large-celled lateral vestibular nucleus (nucleus of Deiters) as found e.g. in the cat (Pompeiano and Brodal 1957b; Brodal et al. 1962; Nyberg-Hansen and Mascitti 1964; Abzug et al. 1973, 1974; Hayes and Rustioni 1981) and in the pigeon (Wold 1978) could not be established in *Varanus exanthematicus*.

Collateralization of descending supraspinal pathways in various vertebrates

A double retrograde tracer study (HRP / ^{3}H-apo-HRP) in the cat (Hayes and Rustioni 1981), showed that all major descending pathways to the spinal cord contain branching axons, influencing widely separate levels of the spinal cord, such as the cervical and lumbar enlargements. The present study in a lizard, *Varanus exanthematicus*, adduces evidence that collateralization of descending supraspinal pathways is a common feature in vertebrates. The degree of collateralization in the various descending pathways ranges from 0% to 50%, in *Varanus exanthematicus* (present study) as well as in mammals (Abzug et al. 1973, 1974; Peterson et al. 1975, 1979; Hayes and Rustioni 1981; Huisman et al. 1981, 1982; Martin et al. 1981a,b). In mammals the degree of collateralization appears to be higher in the medial system of descending brain stem pathways as advocated by Kuypers (see e.g. Kuypers 1964, 1981, 1982) (i.e. reticulospinal, interstitiospinal and vestibulospinal pathways) than in lateral brain stem pathways, as the rubrospinal tract and the spinal projection from the ventrolateral pontine tegmentum (Shinoda et al. 1977; Huisman et al. 1981, 1982; Kuypers 1982), and the corticospinal tract (Shinoda et al. 1977). It has been suggested (Huisman et al. 1982), that in species, in which a somatotopic arrangement of the rubrospinal projection is lacking, as e.g. the pigeon (Wild et al. 1979) and reptiles (ten Donkelaar et al. 1980; Woodson and Künzle 1982; present study), the rubrospinal tract displays an even more pronounced collateralization than in opossum. This assumption cannot be confirmed by the present study, which shows that the degree of collateralization in a lizard rubrospinal pathway (± 25% collaterals beyond the midthoracic level) exceeds that in cat and monkey (± 10%), but is lower than in rat (35%) (Huisman et al. 1982). A decrease of rubrospinal collaterals caudalward in the spinal cord as observed in most mammalian species studied, is also present in *Varanus exanthematicus* (25% > 15%).

In contrast to the spinal projections from the nucleus raphe magnus in rat, cat and monkey (Huisman et al. 1981, 1982), which show approximately constant numbers of collaterals throughout the spinal cord (40%, 40% and 60% respectively), the raphespinal pathway in *Varanus exanthematicus* shows a decrease of collaterals caudalward (42% > 26%). It should be noted, however, that the nucleus raphes inferior in *Varanus exanthematicus*, which forms the origin of the raphespinal projection, is most probably comparable to both nucleus raphe magnus and raphe pallidus in mammals (Wolters et al. 1985a). This might explain, at least partly, the different projection patterns in the mammalian and the lizard raphespinal pathways.

In the nucleus reticularis inferior of *Varanus exanthematicus*, %DL was lower than in the caudal medullary reticular formation of the cat (66%)(Peterson et al. 1975), and decreased when NY was implanted in the lumbar enlargement (35% > 20% ipsilaterally). The two subnuclei of the nucleus reticularis inferior, i.e. the pars ventralis and pars lateralis show larger and smaller numbers of collaterals, respectively (table 1; Fig. 12). The nucleus reticularis superior pars lateralis in *Varanus exanthematicus*, probably homologous to the mammalian ventrolateral pontine tegmentum, shows ± 30% spinal collateralization (i.e. lower than in mammals; see Huisman et al. 1981, 1982) and no decrease caudalward.

In mammals it has been inferred that the fiber tracts, known as the medial system of brain stem pathways (Kuypers 1964) (i.e. reticulospinal, vestibulospinal, and interstitiospinal pathways), influence the spinal cord, especially the motoneurons innervating the trunk musculature and the proximal limb muscles, in a global way, giving off numerous collaterals (Huisman et al. 1982; Kuypers 1982). The pathways forming the lateral brain stem system (arising from the ventrolateral pontine tegmentum and the red nucleus) would supply much more focussed information to the lateral motor column innervating the distal musculature of the limbs, showing only a restricted number of collaterals (Kuypers 1982). Our findings in *Varanus exanthematicus* do not support these inferences, but rather seem to suggest variable patterns of spinal collateralization of the various descending brain stem pathways, independent of their funicular trajectories.

Conclusions

The present study has demonstrated, that all descending supraspinal pathways give off collaterals that may influence widely separate levels of the spinal cord. The almost complete absence of a somatotopic organization of these descending pathways is in high contrast to findings in certain nuclei in mammals. The various descending pathways show obvious quantitative differences in the degree of collateralization. Furthermore, two different patterns of collateralization can grossly be discerned: a) a

gradual decrease of spinal collaterals caudalward, which can be interpreted as a certain specificity of such projections, b) a constant number of collateral nerve fibers throughout the spinal cord, to be interpreted as either a non specific or, in contrast, a highly specific system, focussed exclusively on the cervical and lumbar enlargements In order to reveal the meaning of the distinction between both patterns, further investigations would be necessary, for which the multiple retrograde fluorescent tracer technique could be a useful tool

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7

COURSE AND SITE OF TERMINATION OF RAPHESPINAL PATHWAYS IN A LIZARD, VARANUS EXANTHEMATICUS, AS STUDIED WITH THE ANTEROGRADE [^{3}H]LEUCINE TRACING TECHNIQUE

Introduction

Various anterograde degeneration studies in mammals (Rasdolsky 1923; Kuypers et al. 1962; Nyberg-Hansen 1965, 1966; Petras 1967; Martin et al. 1975) and in reptiles (Robinson 1969; Cruce 1975; ten Donkelaar 1976) have shown that most of the descending brain stem pathways to the spinal cord, including projections from the raphe and the adjacent magnocellular reticular formation, terminate in the intermediate zone of the spinal grey matter. These descending supraspinal pathways can be subdivided into a medial and lateral system (Kuypers 1964, 1982), the former terminating in the ventromedial part, the latter in the dorsal and lateral parts of the intermediate zone. A similar projection pattern was also found in reptiles (ten Donkelaar 1976). Recently, studies using modern anterograde tracing techniques (Basbaum et al. 1978; Holstege et al. 1979; Martin et al. 1979a,b, 1981, 1982a,b, 1985; Holstege and Kuypers 1982a,b; Ross et al. 1984; Zemlan et al. 1984) have shown that raphe- and reticulospinal pathways terminate in the laminae I and II of the dorsal horn, directly on motoneurons in the ventral horn, as well as in the autonomic preganglionic intermediolateral cell column. The demonstration of direct reticulospinal projections to spinal motoneurons is in keeping with earlier electrophysiological data in mammals (Lund and Pompeiano 1968; Shapovalov 1972, 1975; Peterson et al. 1978, 1979; Peterson 1979, 1980), as well as in reptiles (Shapovalov 1972, 1975) and amphibians (Shapovalov 1972; Shapovalov and Shirjaev 1973; Cruce 1974). Furthermore, electrophysiological evidence was found for raphespinal inhibition of dorsal horn interneurons (Engberg et al. 1968; Fields et al. 1977; Willis et al. 1977; Belcher et al. 1978), inhibition of spinal reflexes, and excitation as well as inhibition of autonomic preganglionic neurons (see Willis 1984 for review).

These findings all fit in with data from histofluorescence (Dahlstrom and Fuxe 1964, 1965; Fuxe 1965; Bjorklund and Skagerberg 1982) and more recent immunohistochemical studies (Steinbusch 1981; Bowker et al. 1981a,b, 1982, 1983; Ruda et al. 1982; DiTirro et al. 1983; Kojima et al. 1983) on the distribution of serotonin-containing fibers and varicosities in the dorsal and ventral horn of the spinal cord, as well as in the intermediate zone and the intermediolateral cell column. In the reptilian spinal cord a similar distribution pattern of serotonin was found (Ueda et al. 1983; Wolters et al. 1985). Since the serotonergic innervation of the spinal cord is mediated almost entirely by supraspinal serotonergic cell populations (Carlsson et al. 1964; Dahlstrom and Fuxe 1965; Oliveras et al. 1977; LaMotte et al. 1982), as the raphe nuclei and certain parts of the reticular formation, these data strongly suggest the existence of direct raphespinal projections to interneurons of the dorsal horn (see also Miletic et al. 1984), as well as to somatic and autonomic preganglionic motoneurons.

In the present study the anterograde [^{3}H]leucine tracing technique has been used to investigate the projections from the raphe and the adjacent reticular formation to the spinal cord in a lizard, *Varanus exanthematicus*, and to obtain more precise information about the course and site of termination of these pathways.

Materials and techniques

Eight lizards were used in the present study, varying in weight from 650 to 1200 g, with a total length of 63 to 72 cm and a snout-vent length of 31 to 35 cm.

Prior to operation the animals received anaesthesia by inhalation of Ethrane, after which they were intubated; during surgery a mixture of Ethrane, oxygen and nitrous oxide was ad-

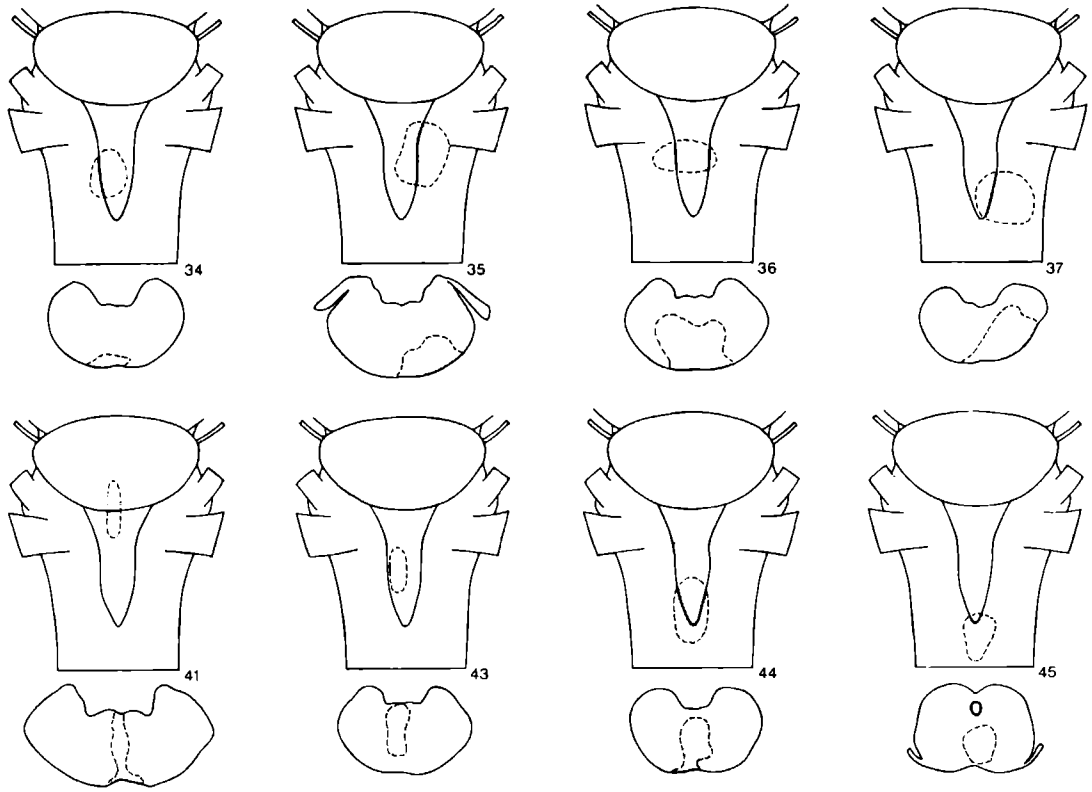


Fig. 1. Dorsal and transversal view of the [^{3}H]leucine injection sites of all cases discussed in the text. Case numbers are indicated.

ministered. The skull was fixed in a stereotact. Under aseptic conditions a median incision of the neck was performed, skin and neck musculature were spread, and the dense connective tissue connecting the skull and the first vertebra was removed. After incision of the meninges a glass micropipette, attached to a Hamilton syringe placed in a micromanipulator, was moved forward into the desired position in the brain stem under microscopic control, i.e. with its tip in the nucleus raphes inferior or the adjacent magnocellular reticular formation. By varying the angle of the micropipette with the horizontal plane different parts of the inferior raphe nucleus could be reached. Then, over a period of 3 to 5 minutes, one injection of 1 μl L-[4,5-^{3}H]leucine (Radiochemical Centre Amersham, UK) with a specific activity of 130 Ci/mmole, containing 85 to 135 μCi/μl, was made. After injection the micropipette was left in place during 10 minutes before being removed.

To obtain labeled fibers and terminal elements throughout the spinal cord survival times of at least 12 weeks were necessary, as well as an environmental temperature ranging from 30 to 35 °C (to increase metabolism and thus the rate of tracer transport). Shorter survival times or lower environmental temperatures resulted in the absence of labeling caudal to high cervical spinal levels. After 12 to 14 weeks the animals were perfused transcardially under deep Nembutal anaesthesia with a 4% formaldehyde solution. The brain and selected segments of the spinal cord were removed, postfixed in 4% formaldehyde during 2 weeks, and subsequently embedded in paraplast (Oxford Laboratories). Subsequently the material was cut on a microtome (AO, Spencer 820) into sections of 7 μm thickness, which were mounted, using distilled water, on chromalum-coated glass slides, and dried overnight at 37 °C. Then the slides were coated with Ilford G5 nuclear emulsion, using a dipping-apparatus with a rate of 96 mm/min and a constant emulsion temperature of 30 °C, dried on an ice-cold metal plate at a relative room humidity of 70% to 90%, and stored in dark boxes containing silicagel, in a lead castle at 4 °C during 12 weeks. The slides were developed with Kodak D-19 high contrast developer (3 minutes, 16 °C), rinsed in distilled water, fixed with 30% sodium thiosulfate (10 minutes), dehydrated and coverslipped with DePeX (Gurr®) as mounting-medium.

The unstained sections were examined at low magnification with a Zeiss operation microscope, using a Wild-Heerbrugg darkfield illumination apparatus. Photographs were made with a Leitz Aristophot under Wild darkfield illumination or with incident skimming light, using Ilford GP4 film.

Results

In five cases the [³H]leucine injection was restricted to the nucleus raphes inferior (Fig. 1 diagrammatically shows the injection sites). Three cases are illustrated in Figs. 2 and 3. One injection was made in the most rostral part of the nucleus raphes inferior at the level of the nucleus reticularis medius (case 41, Fig. 2 A), two injections in its middle part: one dorsal injection (case 43) and one that remained restricted to the ventralmost part of the raphe (case 34), one injection was placed in the raphe at the level of the obex (case 44, Fig. 3 A), and one in the raphe just caudal to the obex (case 45, Fig. 3 B). In three cases the injection site included the adjacent reticular formation. One of these experiments, case 36, is illustrated in Fig. 2 B.

In case 41 (Fig 2 A), i.e. the [³H]leucine injection into the most rostral part of the nucleus reticularis inferior, labeled descending fibers were found bilaterally throughout the peripheral part of the lateral funiculus, particularly in its dorsal part. In addition, abundant bilateral labeling of descending fibers was observed in the medial part of the ventral funiculus. The number of labeled fibers diminishes caudalwards, particularly in the ventral funiculus. In the spinal grey, the labeled fibers were distributed to the superficial layers of the dorsal horn (more or less comparable to areas I and II, according to Kusuma et al. 1979) and to the central parts of the intermediate zone (area X and adjacent parts of areas V to VIII). The labeling in the dorsal part of the dorsal horn was restricted to cervical and thoracic segments, the distribution of labeled elements in the central parts of the intermediate zone was found from the cervical intumescence to the lumbar enlargement. No labeling was observed in the motoneuronal area (area IX).

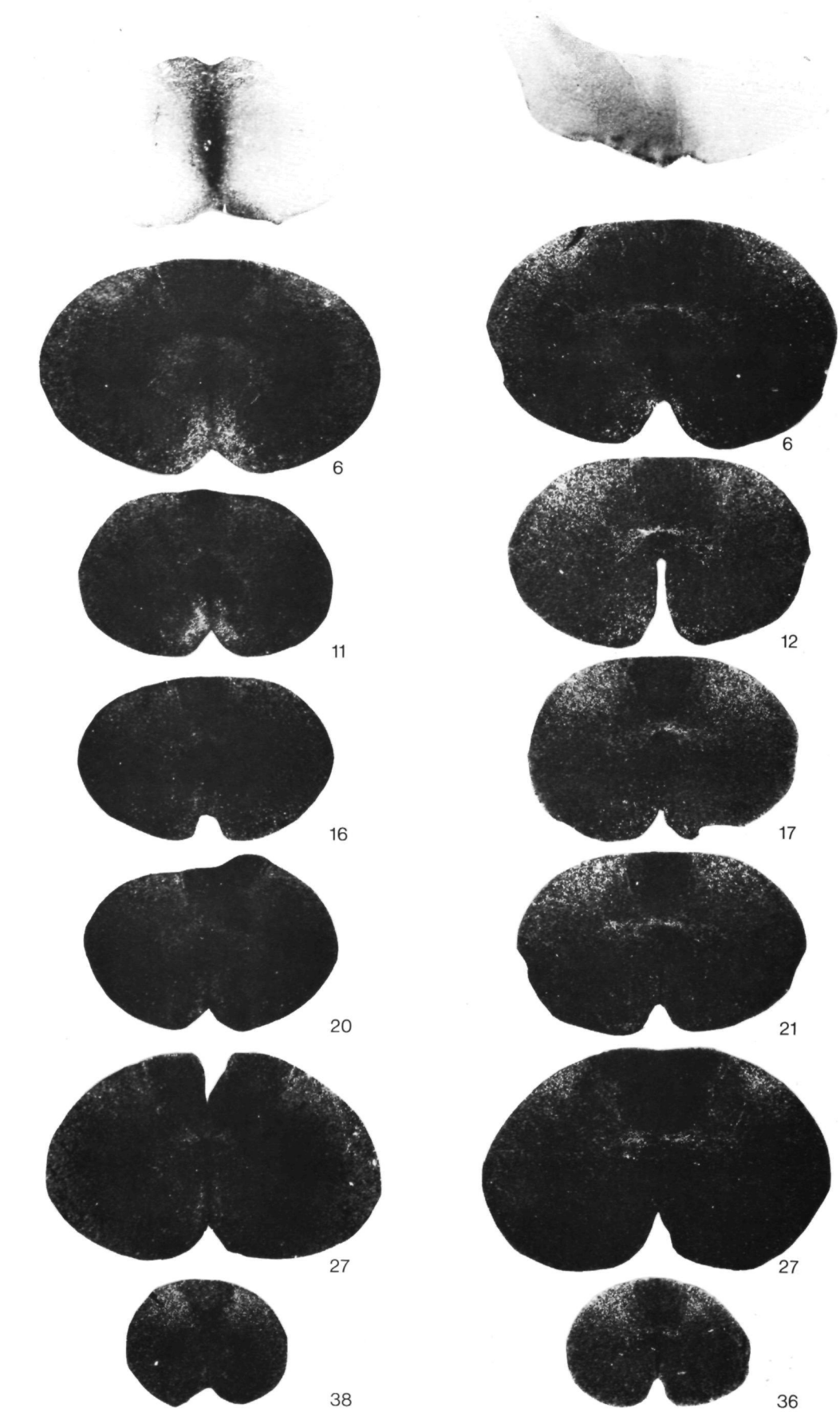
Following a [³H]leucine injection in the middle part of the nucleus raphes inferior (cases 34 and 43) labeled fibers were again distributed bilaterally throughout the lateral funiculus, especially in its dorsal part, but only sparsely in the ventral funiculus. At this level, the dorsal extension of the raphe (case 43) projects almost exclusively via the dorsal part of the lateral funiculus, whereas the ventralmost part of the raphe (case 34) projects via both the dorsal and ventral parts of this funiculus. Particularly abundant labeling was observed in experiment 36, in which case, however, also the adjacent ventral part of the medial reticular field (the rostral part of the nucleus reticularis inferior) was involved. In this experiment (Fig. 2 B), especially the conspicuous labeling of presumably terminal elements in the dorsal horn at caudal thoracic and lumbar levels should be noted. In the same experiment, the distribution of labeled elements in the central part of the intermediate zone extends into the tail cord segments studied. No labeling was present in the motoneuronal area.

Two [³H]leucine injections were made into the caudal part of the nucleus raphes inferior, one rostral to the obex (case 44, Fig. 3 A), and one just caudal to the obex (case 45, Fig. 3 B). In case 44 throughout the length of the spinal cord labeled fibers were found bilaterally in the lateral funiculus, particularly in its dorsal part. In addition, labeled fibers were present in the ventral funiculus at cervical levels, and, more sparsely, at thoracic levels. In this case labeling of the spinal grey matter was restricted to the central and ventromedial parts of the intermediate zone. No labeled elements were found in either the dorsal horn or the motoneuronal area.

In case 45 labeled fibers were found throughout the lateral and ventral funiculi. In the spinal grey matter the most conspicuous labeling was seen in the motoneuronal area throughout the spinal cord, especially in the cervical and lumbar enlargements. In the intermediate zone labeled elements were less numerous, no label at all was found in the dorsal horn. Labeling of elements in the ventral horn,

Fig. 2. Bright-field photomicrographs showing the [³H]leucine injection sites, and dark-field photomicrographs showing the spinal distribution of labeled fibers in selected segments of the spinal cord (numbers indicate the spinal levels of the pictured sections). **A.** High density of labeled fibers in the medial part of the ventral funiculus following an injection restricted to the rostralmost part of the nucleus raphes inferior (case 41), and innervation of the central and ventromedial parts of the intermediate zone, as well as the dorsal part of the dorsal horn at cervical and thoracic levels. Note the absence of labeled elements in the ventrolateral part of the ventral horn, i.e. the motoneuronal area. **B.** High density of labeled fibers in the dorsal part of the lateral funiculus and few labeled fibers in the ventral funiculus following a [³H]leucine injection in the middle part of the nucleus raphes inferior and the adjacent reticular formation (case 36). Labeled elements are distributed to the central part of the intermediate zone throughout the spinal cord. Note the conspicuous innervation of the dorsal horn at low thoracic and lumbar levels.

Fig. 3. Bright-field photomicrographs showing the [³H]leucine injection sites, and dark-field photomicrographs showing the spinal distribution of labeled fibers in selected segments of the spinal cord (numbers indicate the spinal levels of the pictured sections). **A.** Labeled fibers in the lateral funiculus, especially its dorsal part, throughout the spinal cord, and in the ventral funiculus particularly at cervical and high thoracic levels, following an injection in the caudal part of the raphe, rostral to the obex (case 44). Innervation is restricted to the central and ventromedial parts of the intermediate zone. **B.** Labeled fibers troughout the lateral and ventral funiculi, following a [³H]leucine injection in the raphe caudal to the obex. Note the innervation throughout the intermediate zone at all levels of the spinal cord, as well as the conspicuous labeling of the motoneuronal area, especially at the cervical and lumbar enlargements.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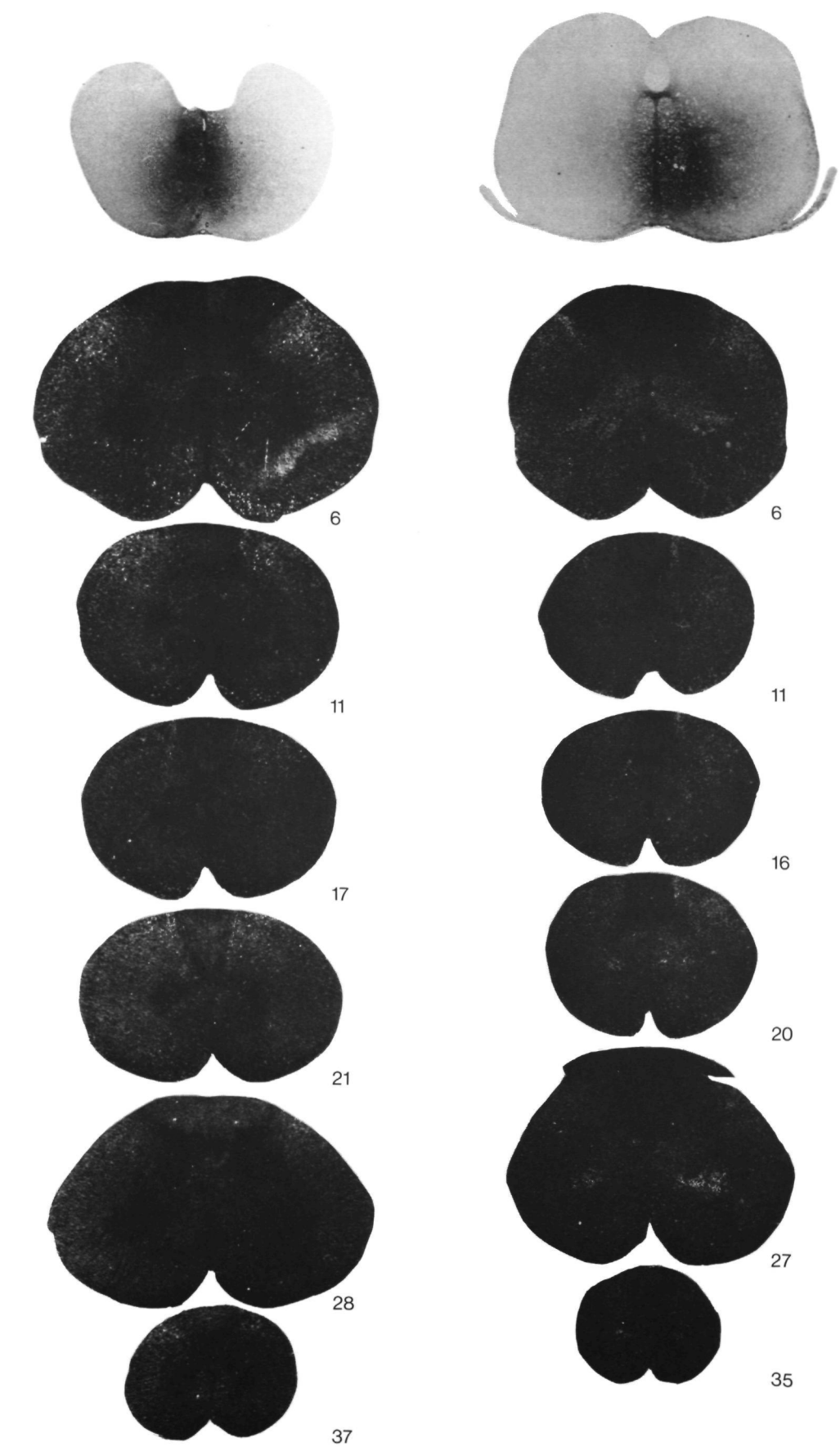
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especially at lumbar levels, was also found in experiment 37, following an injection in the caudal part of the nucleus reticularis inferior, comprising the ventral part of the caudal raphe.

In summary, most parts of the nucleus raphes inferior were found to project to the spinal cord via both the lateral and ventral funiculi. The rostralmost part of the nucleus raphes inferior projects to the spinal cord mainly via the medial part of the ventral funiculus and innervates the central and ventromedial parts of the intermediate zone. In addition the dorsal and lateral parts of the dorsal horn receive a conspicuous innervation from this part of the raphe, at least at cervical and thoracic levels, from fibers descending via the dorsal part of the lateral funiculus. The middle part of the nucleus raphes inferior projects mainly via the lateral funiculus. Only the central part of the intermediate zone is innervated by this part of the raphe. The adjacent reticular formation at the same level (i.e. the most rostral part of the nucleus reticularis inferior) innervates the lateral part of the dorsal horn. Raphespinal projections arising from the caudal part of the nucleus raphes inferior course via the dorsal part of the lateral funiculus and the ventral part of the ventral funiculus, and innervate the central and ventromedial parts of the intermediate zone. In addition the part of the raphe caudal to the obex innervates the motoneuronal area of the ventral horn throughout the spinal cord, but especially at the cervical and lumbar enlargements. A contribution to the innervation of the lumbar motoneuronal area seems to be made by the caudal part of the nucleus reticularis inferior.

Discussion

The distribution pattern of raphespinal pathways as found in the present study is in keeping with immunohistochemical data in *Varanus exanthematicus* (Wolters et al. 1985), showing a similar distribution of serotonin-containing varicosities in the intermediate zone, the dorsal and the ventral horn. The rostral part of the nucleus raphes inferior, most probably comparable with the mammalian nuclei raphes pontis and magnus (Wolters et al. 1985), accounts for the innervation of the dorsal horn and the intermediate zone. The caudal part of the nucleus raphes inferior, comparable to the mammalian nucleus raphes pallidus (Wolters et al. 1985), is responsible for the innervation of both the motoneuronal area of the ventral horn and the intermediate zone. These findings are readily comparable to those in opossum (Martin et al. 1982a,b) rat (Holstege and Kuypers 1982b; Skagerberg and Björklund 1984; Martin et al. 1985), and cat (Basbaum et al. 1978; Holstege and Kuypers 1982a).

The present data on the funicular trajectory of raphespinal pathways in *Varanus exanthematicus* are in keeping with previous data obtained in a study using HRP slow release gels (Wolters et al. 1982), and are also comparable with data in various mammals (Basbaum et al. 1978; Basbaum and Fields 1979; Tohyama et al. 1979; Goode et al. 1980; Björklund and Skagerberg 1982; Martin et al. 1982a,b; Holstege and Kuypers 1982a; Skagerberg and Björklund 1984).

Projections to the mammalian intermediolateral cell column have been demonstrated from the raphe nuclei and parts of the medullary reticular formation (Basbaum et al. 1978; Martin et al. 1979a, 1981, 1982a,b; Holstege and Kuypers 1982a; Ross et al. 1984). In the reptilian spinal cord no intermediolateral cell column is present, but since it is assumed that autonomic preganglionic neurons are present just lateral to the central canal (Kusuma and ten Donkelaar 1979), the labeled elements found in the present study in area X might include an innervation of such neurons, possibly arising from several parts of the raphe and the medullary reticular formation.

The present study has shown that the raphespinal pathways in a lizard seem to be organized in a similar way as in mammals with respect to their origin, their funicular trajectory, and their site of termination. It may illustrate that throughout terrestrial vertebrates the raphespinal system subserves various spinal functions, as e.g. modulation of pain perception, mediation of various autonomic functions, and gain setting of the spinal motor system.

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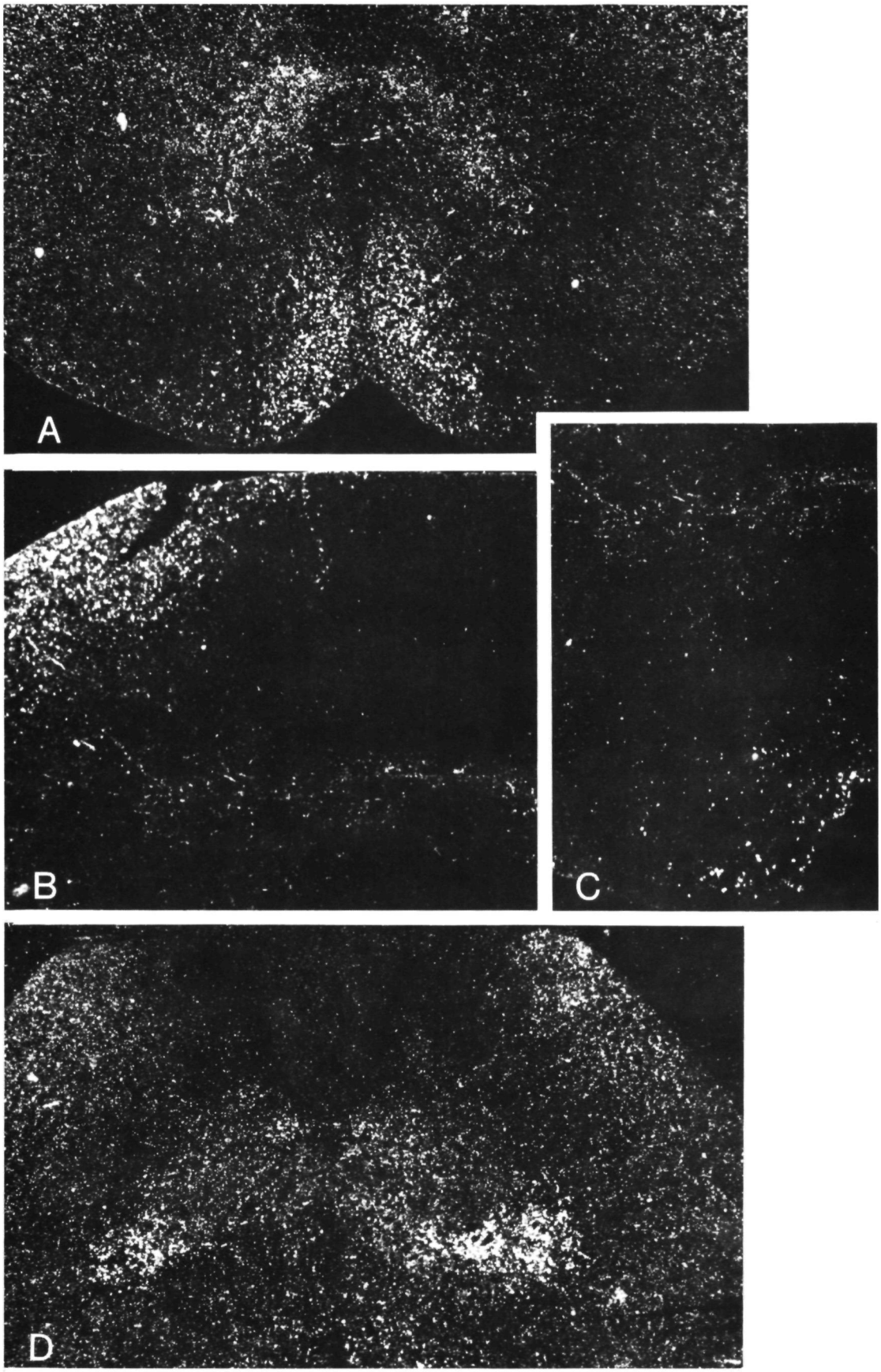
Fig. 4. High magnification photomicrographs. **A.** Case 41, cervical intumescence; labeled fibers in the medial part of the ventral funiculus, and in the dorsal part of the lateral funiculus; labeled elements are distributed to the central and ventromedial parts of the intermediate zone, as well as to the dorsal part of the dorsal horn; note the absence of labeled fibers in the motoneuronal area. **B.** and **C.** Case 36, cervical intumescence; labeled fibers in the dorsal part of the lateral funiculus and in the ventral funiculus, respectively, and innervation of the central part of the intermediate zone. **D.** Case 45, lumbar intumescence; note the conspicuous innervation of the motoneuronal area, as well as the innervation of the entire intermediate zone; labeled descending fibers are present throughout the lateral and ventral funiculi, but particularly in the dorsal part of the lateral funiculus.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APPENDIX

Pascal program, used on an APPLE II microcomputer, for numerical evaluation of the results of Chapter 6

```
(*$C            Copyright Loek Leenen Nijmegen 1984            *)
(*$S+++)
PROGRAM FLUTRACE; uses transcend,turtlegraphics,applestuff;

(*****************************************************************)
(*                                                               *)
(*      Programma om DUBBELE LABELING te evalueren               *)
(*      Versie: 02              Datum:26-12-1984                 *)
(*                                                               *)
(*****************************************************************)

Const X1=30;X2=279;X3=230;X4=130;X5=50;
      Q1=30;Q2=160;Q3=158;Q4=162;Q5=40;
      Q6=60;Q7=80;Q8=110;Q9=90;Q10=140;Q11=130;
      Q12=100;Q13=70;Q14=45;Q15=50;Q16=120;Q17=170;

Type beest= record
                kant: 1..2 ;
                kern: 1..25 ;
                section: 1..50 ;
                NY,FB,DL: 1..41 ;
            end;
     arr = array[1..25,1..50] of integer;
     arr2= array [1..25] of integer;
     arr3= array [1..25] of real;
     arr4= array [1..25,1..7] of integer;
     arr5= array [1..25,1..7] of real;
     arr6= array [1..7] of integer;
     kleintje=array[1..7] of real;

Var zijde: 1..2;
    nucl: 1..25;
    coupe: 1..50 ;
    NY,FB,DL: 1..41 ;
    dier: file of beest ;
    inname: string ;
    ch: char;
    teller: arr2;
    Fast, Yel, Dub: arr4;
    Fabl,Nuye,Dula,PercDL: arr5;
    groep: arr6;
    X:kleintje;
    SDFB,SEFB,SDNY,SENY,SDDL,SEDL,gemFast,gemYel,gemDub:arr3;
    gemPercDL,SDPercDL,SEPercDL:arr3;
    RECNUM,TELLER1,TELLER2,kern,k,n,nr,f,XX: integer;
    Y1,Y2,Y3,Y4,Y5,Y6,Y7,Y8,Y9,Y10,Y11,Y12,Y13,Y14,Y15,Y16,Y17:integer;
    M1G,SD1G,M1G,SD1G,M2G,SD2G,M2G,SD2G: real;
    PRINT                        :INTERACTIVE;
    lumb,aut,graf,klein,Eentje: boolean;
```

```
SEGMENT PROCEDURE GEMGRAF;

    PROCEDURE FACTOR;
    BEGIN
      IF Klein THEN f:=10 ELSE f:=20;
      M10:= gemFast[nucl]*f;
      SD10:=SEFB[nucl]*f;
      M1G:= gemYel[nucl]*f;
      SD1G:=SENY[nucl]*f;
      M20:= gemDub[nucl]*f;
      SD20:=SEDL[nucl]*f;
      M2G:= gemPercDL[nucl]*2;
      SD2G:=SEPercDL[nucl]*2
    END;

    PROCEDURE GRAFIEKRICHTING;
    BEGIN
      IF lumb=false  THEN
      Begin
        Y1:=191-Q1; Y2:=191-Q2; Y3:=191-Q3; Y4:=191-Q4; Y5:=191-Q5;
        Y6:=191-Q6; Y7:=191-Q7; Y8:=191-Q8; Y9:=191-Q9; Y10:=191-Q10;
        Y11:=191-Q11; Y12:=191-Q12; Y13:=191-Q13; Y14:=191-Q14;
        Y15:=191-Q15; Y16:=191-Q16; Y17:=191-Q17
      End
      ELSE
      Begin
        Y1:=Q1; Y2:=Q2; Y3:=Q3; Y4:=Q4; Y5:=Q5; Y6:=Q6; Y7:=Q7;
        Y8:=Q8; Y9:=Q9; Y10:=Q10; Y11:=Q11; Y12:=Q12; Y13:=Q13;
        Y14:=Q14; Y15:=Q15; Y16:=Q16; Y17:=Q17
      End;
    END;

    PROCEDURE ASSENSTELSEL;
    BEGIN
      INITTURTLE;
      PENCOLOR(NONE);
      MOVETO(X1,Y1);
      PENCOLOR(WHITE);
      MOVETO(X1,Y2);
      MOVETO(X2,Y2);
    END;

    PROCEDURE STREEPJES;
    BEGIN
      PENCOLOR(NONE);
      MOVETO(X3,Y3);
      PENCOLOR(WHITE);
      MOVETO(X3,Y17);
      PENCOLOR(NONE);
      MOVETO(X4,Y3);
      PENCOLOR(WHITE);
      MOVETO(X4,Y17);
      XX:=X5;
      WHILE XX<=270 DO
      Begin
        PENCOLOR(NONE);
        MOVETO(XX,Y3);
        PENCOLOR(WHITE);
        MOVETO(XX,Y4);
        XX:=XX + 20;
      End;
    END;
```

```
PROCEDURE INVOER;
BEGIN
  (*1e deel figuur*)
  PENCOLOR(NONE);
  MOVETO(X1,Y10);
  PENCOLOR(WHITE);
  MOVETO(X1+ROUND(M1O),Y10);
  MOVETO(X1+ROUND(M1O),Y11);
  MOVETO(X1+ROUND(M1O+SD1O),Y11);
  MOVETO(X1+ROUND(M1O+SD1O),(Y11+5));
  MOVETO(X1+ROUND(M1O+SD1O),(Y11-5));
  MOVETO(X1+ROUND(M1O+SD1O),Y11);
  MOVETO(X1+ROUND(M1O),Y11);
  MOVETO(X1+ROUND(M1O),Y16);
  MOVETO(X1,Y16);

  (*2e deel figuur*)
  MOVETO(X1,Y8);
  MOVETO(X1+ROUND(M1G),Y8);
  MOVETO(X1+ROUND(M1G),Y12);
  MOVETO(X1+ROUND(M1G+SD1G),Y12);
  MOVETO(X1+ROUND(M1G+SD1G),(Y12+5));
  MOVETO(X1+ROUND(M1G+SD1G),(Y12-5));
  MOVETO(X1+ROUND(M1G+SD1G),Y12);
  MOVETO(X1+ROUND(M1G),Y12);
  MOVETO(X1+ROUND(M1G),Y9);
  MOVETO(X1,Y9);

  (*3e deel figuur*)
  MOVETO(X1,Y7);
  MOVETO(X1+ROUND(M2O),Y7);
  MOVETO(X1+ROUND(M2O),Y13);
  MOVETO(X1+ROUND(M2O+SD2O),Y13);
  MOVETO(X1+ROUND(M2O+SD2O),(Y13+5));
  MOVETO(X1+ROUND(M2O+SD2O),(Y13-5));
  MOVETO(X1+ROUND(M2O+SD2O),Y13);
  MOVETO(X1+ROUND(M2O),Y13);
  MOVETO(X1+ROUND(M2O),Y6);
  MOVETO(X1,Y6);

  (*4e deel figuur*)
  MOVETO(X1,Y15);
  MOVETO(X1+ROUND(M2G),Y15);
  MOVETO(X1+ROUND(M2G),Y14);
  MOVETO(X1+ROUND(M2G+SD2G),Y14);
  MOVETO(X1+ROUND(M2G+SD2G),(Y14+5));
  MOVETO(X1+ROUND(M2G+SD2G),(Y14-5));
  MOVETO(X1+ROUND(M2G+SD2G),Y14);
  MOVETO(X1+ROUND(M2G),Y14);
  MOVETO(X1+ROUND(M2G),Y5);
  MOVETO(X1,Y5);
END;
```

```
PROCEDURE LEGENDS;
BEGIN
  IF lumb THEN
  Begin
    PENCOLOR(NONE);
    IF Klein THEN
    Begin
      MOVETO(X4-6,Y17+4);WSTRING('10');
      MOVETO(X3-6,Y17+4);WSTRING('20')
    End
    ELSE
    Begin
      MOVETO(X4-3,Y17+4);WSTRING('5');
      MOVETO(X3-6,Y17+4);WSTRING('10')
    End;

    PENCOLOR(NONE);
    MOVETO((X4-6),(Y2-12)); WSTRING('50%');
    MOVETO((X3-13),(Y2-12));WSTRING('100%');

    MOVETO(X1+65,Y2+14);  WSTRING('->');
    MOVETO((X1+2),(Y2+4));WSTRING('CELLS/SECTION');
    MOVETO(X1+2,Y2-12);   WSTRING('DL/FB+DL ->');
  End
  ELSE
  Begin
    PENCOLOR(NONE);
    IF Klein THEN
    Begin
      MOVETO(X4-6,Y17-12);WSTRING('10');
      MOVETO(X3-6,Y17-12);WSTRING('20')
    End
    ELSE
    Begin
      MOVETO(X4-3,Y17-12);WSTRING('5');
      MOVETO(X3-6,Y17-12);WSTRING('10')
    End;

    PENCOLOR(NONE);
    MOVETO((X4-6),(Y2+4)); WSTRING('50%');
    MOVETO((X3-13),(Y2+4));WSTRING('100%');

    MOVETO(X1+65,Y2-12);  WSTRING('->');
    MOVETO((X1+2),(Y2-12));WSTRING('CELLS/SECTION');
    MOVETO(X1+2,Y2+4);   WSTRING('DL/FB+DL ->');
  End
END;

PROCEDURE NAMEN;
BEGIN
  PENCOLOR(NONE);
  MOVETO(0,Y11-4);
  WSTRING('FB');
  MOVETO(0,Y12-4);
  WSTRING('NY');
  MOVETO(0,Y13-4);
  WSTRING('DL');
  MOVETO(0,Y14-4);
  WSTRING('%DL');
END;
```

```
PROCEDURE ARCERING;
VAR I:INTEGER;
BEGIN
  IF lumb THEN VIEWPORT(X1,(X1+ROUND(M1O)),Y16,Y1O)
          ELSE VIEWPORT(X1,(X1+ROUND(M1O)),Y1O,Y16);
  FOR I:=X1-20 TO (X1+ROUND(M1G)) DO
    BEGIN
      PENCOLOR(NONE);
      MOVETO(I,Y1O);
      PENCOLOR(WHITE);
      MOVETO(I+20,Y16);
      I:=I+5;
    END;

  IF lumb THEN VIEWPORT(X1,(X1+ROUND(M1G)),Y9,Y8)
          ELSE VIEWPORT(X1,(X1+ROUND(M1G)),Y8,Y9);
  FOR I:=(X1+ROUND(M1G))+20 DOWNTO X1 DO
    BEGIN
      PENCOLOR(NONE);
      MOVETO(I,Y8);
      PENCOLOR(WHITE);
      MOVETO(I-20,Y9);
      I:=I-10;
    END;

  IF lumb THEN
  Begin
    VIEWPORT(X1,(X1+round(M2G)),Y5,Y15);
    FOR I:=(Y15-2) DOWNTO (Y5+2) DO
    BEGIN
      PENCOLOR(NONE);
      MOVETO(X1,I);
      PENCOLOR(WHITE);
      MOVETO(X1+ROUND(M2G),I);
      I:=I-2;
    END;
  End
  ELSE
  Begin
    VIEWPORT(X1,(X1+round(M2G)),Y15,Y5);
    FOR I:=(Y5-2) DOWNTO (Y15+2) DO
    BEGIN
      PENCOLOR(NONE);
      MOVETO(X1,I);
      PENCOLOR(WHITE);
      MOVETO(X1+ROUND(M2G),I);
      I:=I-2;
    END;
  End;
END;

PROCEDURE WACHT;
VAR I:INTEGER;
BEGIN
  REPEAT;
  I:=0;
  FOR I:=0 TO 30 DO
    BEGIN
      I:=I+1;
    END;
  UNTIL KEYPRESS;
END;
```

```
    PROCEDURE PRINTEN;
    BEGIN
      IF aut=false THEN
      Begin
        WACHT;
        TEXTMODE;
        WRITELN;WRITE('Wil je deze grafiek printen ?  (Y/N)   ');
        READ(ch);
        IF ch in ['N', n'] THEN exit(PRINTEN)
      End;
      GRAFMODE;
      WRITELN(PRINT,CHR(25),'G');
      TEXTMODE
    END;

BEGIN
  FACTOR;
  GRAFIEKRICHTING;
  ASSENSTELSEL;
  STREEPJES;
  INVOER;
  LEGENDS;
  NAMEN;
  ARCERING;
  PRINTEN;
END;

PROCEDURE NAAMFRI(S,I:STRING);
BEGIN
  CLOSE(PRINT);
  RESET(PRINT,'PRINTER:');
  WRITELN(PRINT,S:11,'  ',I:14,',');
END;

PROCEDURE OPSOMMEN(KANTEN:INTEGER);
VAR I:STRING;
BEGIN
    CASE KANTEN OF
      1: I:= iosilateraal ;
      2: I:='contralateraal';
    END;
    NAAMFRI(inname,I);
END;

PROCEDURE KERNEN;
BEGIN
  writeln;
  writeln(' 1 = Ra1     6 = Xmd     11 = Vevm     16 = Rs     21 = Rub ');
  writeln(' 2 = Ri      7 = Veds    12 = Vevl     17 = Rsl    22 = Nflm');
  writeln(' 3 = Ril     8 = Vds     13 = Rm       18 = Cerm   23 = Iflm');
  writeln(' 4 = Riv     9 = ...     14 = Rml      19 = Lc     24 = Ph  ');
  writeln(' 5 = Sol    10 = Phg     15 = Vpr      20 = Sc     25 = Alh ');
END;

PROCEDURE KEUZE;
VAR ch:char;

BEGIN
  write ('Slechts 1 dier ?            (Y/N)  ');
  readln(ch);
  IF ch in ['Y','y'] THEN
```

```
  Begin
    Eentje:=true;
    writeln; write('Filenaam  (vol. + diernr.)   :      );
    readln(inname);
    groep[1]:=0
  End ELSE
  Begin
    write ('Hoeveel dieren ?                    );
    readln(n);
    FOR k:=1 to n do
    Begin
      write('Welke dieren ?   (geef alleen het nummer)    ');
      readln(nr);
      groep[k]:=nr
    End;
  End;
  write('Gaat het om een LUMBALE implantatie ?        ');
  read(ch);
  IF ch in ['N', n ] THEN lumb:=false;
  writeln;
  KERNEN
END;

PROCEDURE INLEZEN;
VAR dier: file of beest;
    teller: arr2;
    i:INTEGER;

BEGIN
  (*INITIALISATIE*)
  TELLER1:=0;i:=0; k:=0;

  REPEAT
    k:=k+1;
    CASE groep[k] OF
         0 : inname:=inname;
         1 : inname:='jan3:dier1';
         2 : inname:='jan3:dier2';
         3 : inname:='jan3:dier3';
         4 : inname:='jan3:dier4';
         5 : inname:='jan3:dier5';
         6 : inname:='jan3:dier6';
         7 : inname:='jan3:dier7';
         8 : inname:='jan3:dier8';
         9 : inname:='jan3:dier9';
         10: inname:='jan3:dier10 ;
         11: inname:='jan3:dier11';
         12: inname:='jan3:dier12'
    END;
    OPSOMMEN(ZIJDE);
    TELLER1:=TELLER1+1;
    i:=i+1;
    Fast[nucl,i]:=0;
    Yel[nucl,i]:= 0;
    Dub[nucl,i]:= 0;

    RECNUM:=1;
    (*$I-*)
    reset (dier,inname); teller[nucl]:=0;
    if IORESULT<>0 then
```

```
      begin
        write('Geen file geopend ');
        exit(program);
      end;
    (*$I+*)
    while not eof (dier) do
      Begin
        SEEK(DIER,RECNUM);
        GET(DIER);
        with dier^ do
          Begin
            IF (kant = zijde) AND (kern = nucl) THEN
            BEGIN
              teller[nucl]:= teller[nucl] +1;
              Fast[nucl,i]:= Fast[nucl,i] + FB;
              Yel[nucl,i]:= Yel[nucl,i] + NY;
              Dub[nucl,i]:= Dub[nucl,i] + DL;
            END;
            RECNUM:=RECNUM+1;
          End;
      End;

    IF teller[nucl]<>0 THEN
    BEGIN
      Fabl[nucl,i]:=(Fast[nucl,i]/teller[nucl]);
      Nuye[nucl,i]:=(Yel[nucl,i]/teller[nucl]);
      Dula[nucl,i]:=(Dub[nucl,i]/teller[nucl]);
       IF Dub[nucl,i]+Fast[nucl,i]=0 THEN Fast[nucl,i]:=1;
      PercDL[nucl,i]:=(Dub[nucl,i]/(Dub[nucl,i]+Fast[nucl,i]))*100;
    END
    ELSE WRITELN('NIETS gevonden');
    CLOSE(dier);

    IF Eentje THEN Begin
                     TELLER2:=TELLER1 + 1;
                     exit(INLEZEN)
                   End;

    TELLER2:=TELLER1;

  UNTIL i=n
END;

PROCEDURE HEADLINE;
BEGIN
   WRITELN('************************************************************');
   WRITELN('*                                                          *');
   WRITELN('*               Het FLUTRACE programma                     *');
   WRITELN('*                                                          *');
   WRITELN('*           Programma voor het samenvatten van             *');
   WRITELN('*           groepen dieren ingespoten met ver-             *');
   WRITELN('*           schillende fluorescerende TRACERS,             *');
   WRITELN('*           op verschillende niveaus in het                *');
   WRITELN('*               ruggemerg van de VARAAN                    *');
   WRITELN('*                                                          *');
   WRITELN('************************************************************');
END;
```

```
PROCEDURE COMMENT;
VAR PRINT:INTERACTIVE;
    S,T,U:STRING;
BEGIN
  RESET(PRINT, PRINTER: );
  WRITELN(PRINT);WRITELN(PRINT);WRITELN(PRINT, COMMENTAAR
  WRITELN('Wat is het commentaar ? ');READLN(S);
  WRITELN(PRINT,S);
  WRITELN('Meer commentaar ? ');READLN(T);
  WRITELN(PRINT,T);
  WRITELN('Nog meer commentaar ?');READLN(U);
  WRITELN(PRINT,U);
  WRITELN(PRINT);WRITELN(PRINT)
END;

PROCEDURE arrayvorm(var X:kleintje; hulp:arr5;nucl:integer);
VAR I:INTEGER;
BEGIN
  FOR I:=1 TO TELLER1 DO X[I]:=hulp[nucl,I];
END;

FUNCTION SD(VAR X:kleintje; mean:real):real;
VAR kwadvers,somkwad,versch:real;
    I:integer;
BEGIN
  somkwad:=0;
  FOR I:=1 to TELLER1 do
    BEGIN
      versch:=X[I]-mean;
      kwadvers:=SQR(versch);
      somkwad:=somkwad+kwadvers;
    END;
  SD:=SQRT(somkwad/(TELLER2-1));
END;

PROCEDURE Resbeest (nucl,TELLER1,TELLER2:integer);

var totFast,totYel,totDub,totPercDL:arr3;
    i:integer;

Begin
         totFast[nucl]:= 0;
         totYel[nucl]:= 0;
         totDub[nucl]:= 0;
         totPercDL[nucl]:= 0;
         FOR i:=1 to TELLER1 DO
         Begin
           totFast[nucl]:= totFast[nucl] + Fabl[nucl,i];
           totYel[nucl]:= totYel[nucl] + Nuye[nucl,i];
           totDub[nucl]:= totDub[nucl] + Dula[nucl,i];
           totPercDL[nucl]:= totPercDL[nucl] + PercDL[nucl,i]
         End;


         gemFast[nucl]:=totFast[nucl]/TELLER1;
         arrayvorm(X,Fabl,nucl);
         SDFB[nucl]:=SD(X,gemFast[nucl]);
         SEFB[nucl]:=SDFB[nucl]/(SQRT(TELLER1));
```

```
        gemYel[nucl]:=totYel[nucl]/TELLER1;
        arrayvorm(X,Nuye,nucl);
        SDNY[nucl]:=SD(X,gemYel[nucl]);
        SENY[nucl]:=SDNY[nucl]/SQRT(TELLER1);


        gemDub[nucl]:=totDub[nucl]/TELLER1;
        arrayvorm(X,Dula,nucl);
        SDDL[nucl]:=SD(X,gemDub[nucl]);
        SEDL[nucl]:=SDDL[nucl]/SQRT(TELLER1);


        gemPercDL[nucl]:=totPercDL[nucl]/TELLER1;
        arrayvorm(X,PercDL,nucl);
        SDPercDL[nucl]:=SD(X,gemPercDL[nucl]);
        SEPercDL[nucl]:=SDPercDL[nucl]/SQRT(TELLER1);
End;

PROCEDURE UPDATE(nucl:integer);
VAR PRINT:INTERACTIVE;
BEGIN
 CLOSE(PRINT);
 RESET(PRINT,'PRINTER:');

 WRITELN(PRINT, 'Kern: ', nucl);
 WRITE(PRINT,'Gemiddelde FB: ',gemFast[nucl]:3:1, '     SD: ',SDFB[nucl]:3:1);
 WRITELN(PRINT,'     SE: ',SEFB[nucl]:3:1);
 WRITE(PRINT,'Gemiddelde NY: ',gemYel[nucl]:3:1, '     SD: ',SDNY[nucl]:3:1);
 WRITELN(PRINT,'     SE: ',SENY[nucl]:3:1);
 WRITE(PRINT,'Gemiddelde DL: ',gemDub[nucl]:3:1, '     SD: ',SDDL[nucl]:3:1);
 WRITELN(PRINT,'     SE: ',SEDL[nucl]:3:1);
 WRITE(PRINT,'Percentage DL: ',gemPercDL[nucl]:4:1,' % ,');
 WRITE(PRINT,'     SD: ',SDPercDL[nucl]:4:1,' %');
 WRITELN(PRINT,'     SE: ',SEPercDL[nucl]:4:1,' %');
 WRITELN(PRINT)
END;


BEGIN (* main program *)

  HEADLINE;
  graf:=false; aut:=false; Klein:=false; lumb:=true; Eentje:=false;

  writeln;write ('Wil je grafische output ?   (Y/N)  ');
  read(ch);
  IF ch in ['Y','y'] THEN
  Begin
    graf:=true;
    writeln;write ('Automatisch na elke kern ?  (Y/N)  ');
    read(ch);
    IF ch in ['Y','y'] THEN
    aut:=true;
    writeln;write ('Wil je schaalverkleining (= de grafiek uitrekken) ? ');
    read(ch); writeln; writeln;
    IF ch in ['N','n'] THEN
    Klein:=true
  End ELSE writeln; writeln;
```

```
  KEUZE;
  FOR nucl:=1 to 25 do
  Begin
    FOR zijde:=1 to 2 do
    Begin
      INLEZEN;
      Resbeest(nucl,TELLER1,TELLER2);
      Update(nucl);
      IF graf THEN GEMGRAF
    End;
  End;
  COMMENT
END.
```

SUMMARY

In the present investigation various aspects of the descending pathways from the brain stem to the spinal cord in a lizard, *Varanus exanthematicus*, have been studied with several neuroanatomical tracer techniques and immunohistochemistry.

The horseradish peroxidase (HRP) retrograde tracer technique was used to identify the brain stem nuclei from which descending pathways to the spinal cord arise, and, moreover, to determine the funicular trajectory of the various descending projections in the spinal cord (Chapter 2). Therefore, HRP slow release gels were applied to four different areas of the spinal white matter in the cervical enlargement. Fibers coursing through the dorsal part of the lateral funiculus were found to originate in the contralateral red nucleus, the contralateral nucleus reticularis superior pars lateralis (comparable to the mammalian ventrolateral pontine tegmentum), the ipsilateral locus coeruleus and subcoeruleus, bilaterally in the nucleus reticularis inferior and the nucleus of the solitary tract, and in the nucleus raphes inferior. The ventral part of the lateral funiculus contains hypothalamospinal fibers, and some reticulospinal and coeruleospinal fibers. Most fibers descending via the ventral and medial parts of the ventral funiculus were found to originate in the the contralateral medial cerebellar nucleus, the locus subcoeruleus, the vestibular nuclear complex, and throughout the magnocellular reticular formation, including the nucleus raphes inferior. Interstitiospinal fibers descend via a separate bundle in the dorsalmost part of the ventral funiculus. Although these data thus indicate a clear-cut arrangement of the spinal course of the descending pathways, they suggest a wider funicular distribution of various projections than was observed in earlier studies, which is in keeping with recent findings in mammals.

With the anterograde tracing technique using [^{3}H]leucine as a marker the descending projections from the nucleus raphes inferior and the adjacent magnocellular reticular formation were studied, in order to obtain more detailed information on their funicular course and, especially, on their site of termination in the spinal grey matter at various levels of the spinal cord (Chapter 7). The HRP data on the funicular trajectory of raphespinal projections were largely confirmed. In addition, rostrally originating raphespinal fibers were found passing mainly via the medial part of the ventral funiculus, whereas caudal raphespinal fibers descend in both the ventral and lateral funiculus, but preferentially in the dorsal part of the lateral funiculus. Most parts of the nucleus reticularis inferior and the nucleus raphes inferior give rise to projections terminating in the intermediate zone of grey matter throughout the length of the spinal cord. From the rostralmost part of the nucleus raphes inferior a distinct projection to the dorsal horn arises, reaching at least as far as low thoracic spinal levels. The lumbar dorsal horn receives an input from the rostralmost part of the nucleus reticularis inferior. Projections terminating in the motoneuronal area of the ventral horn throughout the spinal cord, but especially at the level of the cervical and lumbar enlargements, arise in the caudalmost part of the raphe, caudal to the obex. A relatively sparse projection to lumbar motoneurons, originating in the caudal part of the reticular formation, seems likely. Although the anterograde [^{3}H]leucine technique can be employed successfully in reptiles, experience is still limited. Therefore, only positive results should be taken into account. So far these results are largely in keeping with data in various mammals.

The branching pattern of the descending supraspinal pathways was studied with the multiple retrograde fluorescent tracer technique (Chapter 6). Four questions have been approached: 1) Do the descending brain stem pathways give off collaterals that may influence widely separate levels of the spinal cord ? 2) Is there evidence for a somatotopic arrangement within the brain stem projections to the spinal cord ? 3) Are there quantitative differences in the degree of collateralization of different descending brain stem pathways ? 4) Are there differences in the distribution of collaterals throughout the spinal cord between the different descending pathways ? To tackle these problems "Fast Blue" (FB) gels were implanted unilaterally in the spinal grey matter of the cervical enlargement, whereas "Nuclear Yellow" (NY) gels were implanted, ipsilaterally, in all spinal funiculi of the lumbar enlargement or in midthoracic spinal segments, in two consecutive series of experiments, respectively. Following these tracer applications, all brain stem and hypothalamic nuclei known to project to the spinal cord in reptiles were found to contain neurons, single-labeled with either FB or NY, as well as double-labeled FB-NY neurons. The amount of double-labeled (DL) cells

varied, in the 23 brain stem nuclei studied, from 0 to 50% of the number of neurons projecting to the cervical enlargement. Highly collateralizing projections were found to arise from the ipsilateral nucleus periventricularis hypothalami, the nucleus reticularis superior, the nucleus reticularis superior pars lateralis, the contralateral nucleus princeps nervi trigemini, the contralateral nuclei vestibulares ventromedialis and descendens, the nucleus reticularis inferior pars ventralis, and the nucleus raphes inferior. In contrast to findings in mammals, e.g. with respect to the red nucleus, the nucleus vestibularis ventrolateralis and certain parts of the reticular formation, none of the projections studied in *Varanus exanthematicus* with the multiple fluorescent retrograde tracer technique displayed a clear-cut somatotopic organization. In general two different patterns of collateralization could be distinguished: 1) the amount of spinal collaterals is approximately constant beyond the midthoracic spinal level; 2) the amount of spinal collaterals is gradually decreasing caudalwards. The latter distribution pattern of branching fibers is found in projections from e.g. the ipsilateral nucleus periventricularis hypothalami, the nucleus interstitialis of the fasciculus longitudinalis medialis, the nucleus ruber, the contralateral nucleus cerebellaris medialis, the nucleus princeps nervi trigemini, the nuclei vestibulares ventrolateralis and descendens, the ipsilateral nucleus vestibularis ventromedialis, the nucleus prepositus hypoglossi, the nucleus tractus solitarii, the nucleus reticularis inferior pars lateralis, the ipsilateral nucleus reticularis inferior and the nucleus raphes inferior. These data, however, do not allow conclusions with respect to the specificity (i.e. projections to rather restricted segments of the spinal cord, or global information directed to a wide range of spinal segments) of the various descending pathways.

Apart from further investigations on the nuclei of origin, the course, and site of termination of the descending pathways from the brain stem to the spinal cord, the present study has made an attempt to reveal the nature of part of these projections with respect to their (putative) neurotransmitter content. Therefore the distribution of catecholamines (Chapter 3), serotonin (Chapter 4), and some peptides (substance P, [Leu]- and [Met]enkephalin, Chapter 5) in the brain stem and spinal cord of *Varanus exanthematicus* has been studied with the indirect immunofluorescence technique, using antibodies to tyrosine hydroxylase, serotonin, and the three peptides mentioned, respectively.

The following nuclei, giving rise to spinal projections, were found to contain catecholaminergic cell bodies: the lateral hypothalamic area, the laminar nucleus of the torus semicircularis, the locus coeruleus and subcoeruleus, the nucleus of the solitary tract, and a lateral part of the nucleus reticularis inferior. Descending catecholaminergic fibers to the spinal cord were found in the dorsomedial part of the lateral funiculus, and appeared to terminate mainly in the dorsal horn. Apart from these data related to the descending supraspinal pathways, two important catecholaminergic cell groups were found which are known to project rostralwards, viz., the substantia nigra and the ventral tegmental area. From these cell groups, as well as from the dorsal isthmic tegmentum, ascending catecholaminergic fibers could be traced through the lateral hypothalamus, corresponding to the mesostriatal and isthmocortical projections, respectively, as described in mammals. Furthermore, a few catecholaminergic neurons were found in the spinal cord, just ventral to the central canal. These intrinsic bipolar catecholaminergic neurons contact the cerebrospinal fluid, and apparently do not contribute to the catecholaminergic innervation of the spinal grey matter.

Serotonergic neurons were found predominantly in the midline of the brain stem, i.e. in the nuclei raphes superior and inferior. Of these two, only the latter nucleus gives rise to a spinal projection. In addition, considerable amounts of serotonergic neurons were found more laterally at three levels of the brain stem, i.e. in the caudal mesencephalic tegmentum, the isthmic level, and throughout the medulla oblongata. The latter two locations comprise the ventral parts of the nuclei reticulares superior, medius and inferior, from where projections to the spinal cord have been shown to arise. Descending serotonergic fibers could be traced into the spinal cord via the dorsal part of the lateral funiculus, and the ventral and medial part of the ventral funiculus. They were found to innervate mainly three parts of the spinal grey matter throughout the spinal cord, i.e. the dorsal part of the dorsal horn, several parts of the intermediate zone, and the motoneuronal area in the ventral horn. These findings are in keeping with those obtained following [^{3}H]leucine injections in various parts of the nucleus raphes inferior and the adjacent reticular formation.

Substance P-containing neurons were found in a number of brain stem nuclei known to project to the spinal cord: the lateral hypothalamic area, the nucleus periventricularis hypothalami, the nucleus of the fasciculus longitudinalis medialis, the descending nucleus of the trigeminal nerve, the nucleus of the solitary tract, and scattered throughout the medullary reticular formation. Furthermore, substance P-containing cell bodies were present in the nucleus interpeduncularis, and in the trigeminal and spinal ganglia. In the spinal cord, substance P-containing dorsal root fibers can be seen entering the dorsal horn, and innervating especially the areas I and II; some of these fibers can be traced to the dorsolateral part of area X, that surrounds the central canal, where a second, less densely innervated terminal field is seen. Considering the absence of

substance P-containing fibers in the spinal funiculi, the contribution of supraspinal substance P-containing neurons to the substance P innervation of the spinal cord most probably is negligible

Only minor differences exist with respect to the distribution of [Leu]- and [Met]enkephalinergic neurons in the brain stem, as well as to the distribution of fibers and terminal fields of both peptides Most enkephalinergic cell bodies were found in the caudal hypothalamus, i e in the nuclei ventralis and periventricularis hypothalami Less extensive populations of enkephalinergic neurons were present in the nucleus reticularis superior pars lateralis, the vestibular nuclear complex, in and around the descending nucleus of the trigeminal nerve, in the nucleus of the solitary tract, and scattered throughout the medullary reticular formation Most of the nuclei mentioned give rise to spinal projections and thus may participate in the enkephalinergic innervation of the spinal cord In the spinal cord enkephalinergic fibers were found throughout the lateral funiculus, terminating particularly in the dorsal horn (areas I and II) and around the central canal

The immunohistochemical data obtained in the present study thus support evidence for the existence of catecholaminergic, serotonergic, and peptidergic brain stem projections to the spinal cord They do not, however, supply direct evidence for these connections Therefore experiments combining tracer and immunohistochemical or histofluorescence techniques, or the use of specific antibodies as tracers would have been necessary Our numerous attempts to combine the fluorescent retrograde tracer techniques, using "Fast Blue", "True Blue", and "Nuclear Yellow" as tracers, with the indirect immunofluorescence technique, remained unsuccessful, thus leaving the ultimate question on the neurochemical nature of the descending pathways unanswered

Finally, in accordance with experimental data in mammals, we may distinguish the following main groups of descending pathways in *Varanus exanthematicus* 1) A medial group, comprising interstitiospinal, vestibulospinal, cerebellospinal, and reticulospinal, including part of the raphespinal projections, which descend via the ventral funiculus of the spinal cord, and terminate in the central and ventromedial parts of the intermediate zone This group of descending pathways mainly supplies information for trunk and proximal limb musculature 2) A lateral group, consisting of the projections from the contralateral nucleus ruber and the contralateral nucleus reticularis superior pars lateralis, as well as raphespinal projections, which descend via the dorsal part of the lateral funiculus, and terminate in the central and dorsolateral parts of the intermediate zone This group of descending supraspinal pathways in particular supplies the information for distal limb muscles 3) A third group, mainly originating in the presumably dopaminergic, possibly also peptidergic cells of the hypothalamus, the noradrenergic locus coeruleus, the serotonergic nucleus raphes inferior and certain (aminergic and peptidergic) parts of the reticular formation, descending via the lateral funiculus, and terminating directly on preganglionic autonomic neurons as well as somatic motoneurons This third group of descending pathways probably plays an important role in mediating various autonomic responses, and are likely to add motivational drive in motor behaviour, since most of its origins receive an input from limbic structures 4) Furthermore, the projections to the dorsal horn, arising from serotonergic raphe neurons, from noradrenergic neurons in the locus coeruleus and subcoeruleus, and presumably also in certain parts of the nucleus reticularis inferior, as well as from several peptidergic brain stem neurons should be mentioned These pathways may play an important role, together with the substance P input from the spinal ganglia, in regulation of pain perception

SAMENVATTING

Dit proefschrift vormt het verslag van een onderzoek naar verschillende aspecten van uit de hersenstam afdalende zenuwbanen naar het ruggemerg, zoals dat gedaan is aan een reptiel, de hagedis *Varanus exanthematicus*, met behulp van een aantal experimentele neuroanatomische tracer technieken en immunohistochemie

Om te onderzoeken vanuit welke kernen in de hersenstam projecties naar het ruggemerg ontspringen, en vooral, om het traject dat deze banen in het ruggemerg volgen nader te bestuderen, is gebruik gemaakt van het retrograad transport van HRP (het enzym horseradish peroxidase, d w z mierikswortel peroxidase) (Hoofdstuk 2) Daartoe werd HRP, verwerkt in zeer kleine staafjes gel, in verschillende experimenten op verschillende plaatsen in de vezelbanen van het ruggemerg aangebracht Vezels die verlopen door het dorsale deel van de zijstreng bleken hun oorsprong te vinden in de contralaterale nucleus ruber, de contralaterale nucleus reticularis superior pars lateralis (vergelijkbaar met de ventrolateral pontine area in zoogdieren), de ipsilaterale locus coeruleus en subcoeruleus, bilateraal in de nucleus reticularis inferior en de nucleus tractus solitarii, en in de nucleus raphes inferior Het ventrale deel van de zijstreng bleek afdalende vezels uit de hypothalamus te bevatten, alsmede enige afkomstig uit de reticulaire formatie en uit de locus coeruleus De meeste vezels, afdalend via het ventrale deel van de voorstreng, vonden hun oorsprong in de contralaterale nucleus cerebellaris medialis, de locus coeruleus, het vestibulaire kerncomplex, en in het gehele grootcellige deel van de reticulaire formatie, de nucleus raphes inferior niet uitgezonderd Interstitiospinale vezels bleken een geheel eigen traject te volgen, via een aparte bundel in het dorsale deel van de voorstreng Uit deze gegevens blijkt dus een duidelijke ordening van de afdalende banen in hun verloop door het ruggemerg, die echter, zoals ook recentelijk bij zoogdieren is gevonden, over grotere delen van de witte stof van het ruggemerg verspreid zijn dan in vroegere studies was waargenomen

Met de anterograde tracer techniek waarbij gebruik gemaakt wordt van getritieerd leucine ([^{3}H]leucine) als tracer, werden de projecties van de nucleus raphes inferior en de aangrenzende magnocellulaire reticulaire formatie bestudeerd, om meer gedetailleerde informatie te verkrijgen over het funiculair traject van deze vezels, en vooral over hun plaats van eindiging in de grijze stof op verschillende niveaus in het ruggemerg (Hoofdstuk 7) De resultaten van dit onderdeel van onze studie bevestigden grotendeels de gegevens uit het HRP onderzoek (Hoofdstuk 2) aangaande het funiculaire traject van de raphespina le verbindingen Bovendien bleek, dat vezels die ont springen in het rostrale deel van de nucleus raphes inferior, voornamelijk via het mediale deel van de voorstrengen door het ruggemerg lopen, terwijl de vezels die in het achterste deel van de raphe hun oor sprong vinden afdalen via zowel de zijstrengen als de voorstrengen, maar voornamelijk via het dorsale deel van de zijstrengen De meeste delen van de nu cleus raphes inferior en de nucleus reticularis inferi or vormen de oorsprong van projecties, die eindigen in de zona intermedia van de grijze stof over de hele lengte van het ruggemerg Uit het voorste deel van de nucleus raphes inferior ontspringt een duidelijke projectie naar de dorsale hoorn, die minstens reikt tot laagthoracale niveaus van het ruggemerg De dorsale hoorn op lumbaal niveau ontvangt vezels uit het voorste deel van de nucleus reticularis inferior Vezels, eindigend in de voorhoorn op motoneuronen door het hele ruggemerg heen, maar vooral op die van de voor- en achterpoten, zijn afkomstig uit het achterste deel van de raphe, caudaal van de obex Een bescheiden projectie van het caudale deel van de reticulaire formatie naar lumbale motoneuronen lijkt waarschijnlijk Hoewel de anterograde [^{3}H]leucine tracer techniek met succes kan worden aangewend ook in koudbloedige dieren, zoals reptielen, is hiermee nog slechts weinig ervaring opgedaan Daarom mogen alleen positieve resultaten in overweging worden genomen Tot dusver zijn de verkregen gegevens grotendeels in overeenstemming met wat in verschillende zoogdieren is gevonden

Het vertakkingspatroon in het ruggemerg van de afdalende zenuwbanen uit de hersenstam is onderzocht met behulp van de zgn multiple retrograde fluorescerende tracer techniek (Hoofdstuk 6) De vraagstelling viel in vier delen uiteen 1) Geven de uit de hersenstam afdalende banen zijtakken (collateralen) af die ver uit elkaar gelegen delen van het ruggemerg zouden kunnen beinvloeden ? 2) Bestaat er een somatotopische ordening van projecties vanuit de hersenstam naar het ruggemerg ? 3) Zijn er kwantitatieve verschillen in de mate van collateralisatie van de verschillende afdalende banen ? 4) Zijn er verschillen in de verdeling van collateralen over het ruggemerg tussen de verschillende afdalende banen ?

Om een antwoord op deze vragen te vinden werden "Fast Blue" (FB) gels eenzijdig geïmplanteerd in de grijze stof van de cervicale intumescentie, terwijl "Nuclear Yellow" (NY) gels werden geïmplanteerd in alle vezelbundels van het ruggemerg aan dezelfde zijde, in een eerste serie experimenten in de lumbale intumescentie, in een tweede serie in midthoracale segmenten Na het aanbrengen van beide tracers in het ruggemerg bij een dier, bleken er steeds in alle kernen in de hersenstam en de hypothalamus waarvan bekend was dat ze naar het ruggemerg projecteren, zenuwcellen aanwezig te zijn die hetzij FB, hetzij NY, hetzij beide tracers hadden gestapeld Het aantal dubbel-gelabelde (DL) cellen varieerde, in de 23 hersenstamkernen die bestudeerd zijn, van 0 tot 50% van het aantal neuronen dat naar de cervicale intumescentie projecteerde Grote aantallen collateralen bleken te worden afgegeven door vezels waarvan de oorsprongscellen liggen in de ipsilaterale nucleus periventricularis hypothalami, de nucleus reticularis superior, de nucleus reticularis superior pars lateralis, de contralaterale nucleus princeps nervi trigemini, de contralaterale nuclei vestibulares ventromedialis en descendens, de nucleus reticularis inferior pars ventralis, en de nucleus raphes inferior In tegenstelling tot wat bij zoogdieren is gevonden, bv met betrekking tot de nucleus ruber, de nucleus vestibularis ventrolateralis en bepaalde delen van de reticulaire formatie, bleek geen van de door ons met behulp van de multipele retrograde fluorescerende tracer techniek onderzochte afdalende verbindingen in *Varanus exanthematicus* een duidelijke somatotopische ordening te vertonen In het algemeen konden twee verschillende vertakkingspatronen worden onderscheiden 1) het aantal zijtakken van een afdalende baan dat aanwezig is in het ruggemerg blijft tussen de midthoracale en lumbale ruggemergssegmenten in vrijwel constant, 2) het aantal zijtakken van een afdalende baan vermindert gaandeweg in het ruggemerg Dit laatste patroon werd gevonden in projecties vanuit bv de ipsilaterale nucleus periventricularis hypothalami, de nucleus interstitialis van de fasciculus longitudinalis medialis, de nucleus ruber, de contralaterale nucleus cerebellaris medialis, de nucleus princeps nervi trigemini, de nuclei vestibulares ventrolateralis en descendens, de ipsilaterale nucleus vestibularis ventromedialis, de nucleus prepositus hypoglossi, de nucleus tractus solitarii, de nucleus reticularis inferior pars lateralis, de ipsilaterale nucleus reticularis inferior en de nucleus raphes inferior Deze gegevens laten echter geen gevolgtrekkingen toe met betrekking tot de specificiteit (d w z de mate waarin projecties gericht zijn op slechts een beperkt aantal segmenten van het ruggemerg, in tegenstelling tot globale informatie die een groot aantal ruggemergssegmenten bereikt) van de verschillende afdalende banen

In aanvulling op verder onderzoek naar de oorsprongsgebieden, het verloop, en de plaats van eindiging van de uit de hersenstam naar het ruggemerg afdalende banen, is in deze studie een poging ondernomen een aantal van deze verbindingen te karakteriseren met betrekking tot de (vermeende) neurotransmitters die zij bevatten Zo is, met behulp van de indirekte immunofluorescentie techniek, de aanwezigheid en de verspreiding van catecholaminen (Hoofdstuk 3), serotonine (Hoofdstuk 4) en enkele peptiden (substance P, [Leu]enkephaline en [Met]-enkephaline, Hoofdstuk 5) in de hersenstam, de caudale hypothalamus, en het ruggemerg bestudeerd, waarbij antilichamen werden gebruikt tegen resp tyrosine hydroxylase, serotonine, en de drie genoemde peptiden

De volgende kernen, die ook de oorsprong vormen van projecties naar het ruggemerg, bleken catecholaminerge zenuwcellen te bevatten de area lateralis hypothalami, de nucleus laminaris van de torus semicircularis, de locus coeruleus en subcoeruleus, de nucleus tractus solitarii, en een lateraal deel van de nucleus reticularis inferior Afdalende catecholaminerge vezels werden gezien in het dorsomediale deel van de zijstrengen, en bleken voornamelijk in de achterhoorn te eindigen Los van deze gegevens die betrekking hebben op afdalende banen naar het rug gemerg, werden twee belangrijke catecholaminerge celgroepen gevonden die de oorsprong vormen van rostrale projecties, nl de substantia nigra en de area tegmentalis ventralis Vanuit deze twee celgroepen, maar ook vanuit de locus coeruleus en subcoeruleus, konden opstijgende catecholaminerge vezels worden gevolgd door de laterale hypothalamus heen, overeenkomend met resp de mesostriatale en de isthmocorticale projecties zoals die in zoogdieren zijn beschreven Bovendien bleken er ook in het ruggemerg, vlak onder het centrale kanaal, catecholaminerge neuronen aanwezig, zij het slechts weinige Deze bipolaire cellen maken kontakt met de cerebrospinale vloeistof, en lijken geen bijdrage te leveren aan de catecholaminerge innervatie van de grijze stof van het ruggemerg

Serotonine-bevattende zenuwcellen werden voornamelijk gevonden in en vlak naast de mediaanlijn in de hersenstam, d.w z in de nuclei raphes superior en inferior Van deze twee vormt alleen de laatste een oorsprongsgebied van projecties naar het ruggemerg Ook aanzienlijke aantallen serotonerge neuronen bleken meer lateraal in de hersenstam te liggen, en wel op drie niveaus, nl in het caudale deel van het tegmentum mesencephali, in het isthmus gebied, en in vrijwel het gehele verlengde merg De laatste twee plaatsen omvatten ook de ventrale delen van de nuclei reticulares superior, medius en inferior, van waaruit projecties naar het ruggemerg ontspringen In het ruggemerg konden afdalende serotonerge vezels gevolgd worden in het dorsale deel van de zijstrengen, en de ventrale en mediale delen van de voorstrengen Zij bleken voornamelijk drie delen van de grijze stof over de gehele lengte van het ruggemerg te innerveren, nl het dorsale deel van de achterhoorn, verschillende delen van de zona inter-

media, en de plaats waar de motoneuronen gelegen zijn in de voorhoorn Deze resultaten zijn in overeenstemming met de gegevens die voortkwamen uit de [³H]leucine injecties in verschillende delen van de nucleus raphes inferior en de aangrenzende reticulaire formatie

Substance P-bevattende neuronen werden gevonden in een aantal kernen in de hersenstam en de hypothalamus die naar het ruggemerg projecteren, zoals de area lateralis hypothalami, de nucleus periventricularis hypothalami, de nucleus van de fasciculus longitudinalis medialis, de nucleus descendens nervi trigemini, de nucleus tractus solitarii, en verspreid door de reticulaire formatie in het verlengde merg Verder waren er substance P-bevattende neuronen aanwezig in de nucleus interpeduncularis, in het ganglion van de nervus trigeminus, en in de spinale ganglia Vanuit deze laatste ganglia konden substance P-bevattende vezels gevolgd worden door de achterwortel, het ruggemerg in, waar zij voornamelijk de areas I en II van de achterhoorn innerveerden, sommige vezels konden van hieruit verder gevolgd worden naar het dorsolaterale deel van area X, die het centrale kanaal omgeeft, waar zich een tweede, minder dicht geinnerveerd eindigingsgebied bevond Aangezien er geen substance P-bevattende vezels gevonden werden in de witte stof van het ruggemerg, mogen we concluderen dat de bijdrage van substance P-bevattende neuronen in de hersenstam (of hoger) aan de substance P innervatie van het ruggemerg zeer waarschijnlijk slechts gering zal zijn

Met betrekking tot de verspreiding van enerzijds [Leu]- en anderzijds [Met]enkefalinerge neuronen, vezels en eindigingsgebieden in de hersenstam, de caudale hypothalamus en het ruggemerg bestaan slechts geringe verschillen De meeste enkefalinerge neuronen werden gevonden in de caudale hypothalamus, nl in de nuclei ventralis en periventricularis hypothalami Minder talrijk waren de enkefalinerge neuronen in de nucleus reticularis pars lateralis, het vestibulaire kernkomplex, in en rond de nucleus descendens nervi trigemini, de nucleus tractus solitarii, en verspreid in de reticulaire formatie in het verlengde merg. De meeste van deze kernen projecteren naar het ruggemerg, en zouden dus (mede) verantwoordelijk kunnen zijn voor de enkefalinerge innervatie van het ruggemerg In het ruggemerg werden enkefalinerge vezels aangetroffen verspreid door de zijstrengen, en eindigend voornamelijk in de achterhoorn (areas I en II) en rond het centrale kanaal

Deze gegevens, verkregen uit het immunohistochemisch onderdeel van onze studie, ondersteunen het bestaan van catecholaminerge, serotonerge en peptiderge projecties van de hersenstam naar het ruggemerg Zij leveren er echter niet het strikte bewijs voor Daarvoor zouden experimenten die zowel tracer technieken als immunohistochemie of histofluorescentie kombineren, noodzakelijk geweest zijn Onze talrijke pogingen om de fluorescerende retrograde tracers, zoals "Fast Blue", "True Blue" en "Nuclear Yellow", te kombineren met de indirekte immunofluorescentie techniek zijn zonder resultaat gebleven, zodat de uiteindelijke vraag naar de neurochemische aard van de afdalende banen vanuit de hersenstam in *Varanus exanthematicus* open moet blijven

Ten slotte kunnen we, in overeenstemming met experimentele gegevens in zoogdieren, een onderscheid maken tussen de volgende belangrijkste groepen van afdalende verbindingen in *Varanus exanthematicus* 1) Een mediale groep, samengesteld uit interstitiospinale, vestibulospinale, cerebellospinale, en reticulospinale (waaronder raphespinale) projecties, die afdalen via de voorstrengen van het ruggemerg, en eindigen in de centrale en ventromediale delen van de zona intermedia Deze groep afdalende banen bestuurt via het ruggemerg voornamelijk de romp-musculatuur en de proximale spieren van de extremiteiten 2) Een laterale groep, bestaande uit projecties van de contralaterale nucleus ruber en de contralaterale nucleus reticularis superior pars lateralis, maar ook uit raphespinale projecties Zij verlopen door het ruggemerg via het dorsale deel van de zijstrengen, en eindigen in de centrale en dorsolaterale delen van de zona intermedia Deze groep afdalende banen levert voornamelijk informatie voor de distale spieren van de extremiteiten 3) Een derde groep, die voornamelijk zijn oorsprong vindt in de vermoedelijk dopaminerge, misschien ook de peptiderge neuronen van de caudale hypothalamus, de noradrenerge locus coeruleus, de serotonerge nucleus raphes inferior, en in bepaalde (aminerge en peptiderge) delen van de reticulaire formatie Deze banen dalen af via de zijstrengen, en eindigen direkt op preganglionaire autonome neuronen en op motoneuronen in de voorhoorn Deze derde groep van afdalende verbindingen speelt waarschijnlijk een belangrijke rol in de regulatie van verschillende autonome reacties, en lijkt ook voor de motorische uiting van de motivationele drijfveer in het gedrag verantwoordelijk te zijn, want de meeste oorsprongsgebieden van deze groep ontvangen rechtstreekse informatie uit limbische strukturen 4) Verder moet nog de groep genoemd worden van projecties naar de achterhoorn, die zijn oorsprong vindt in de serotonerge raphe cellen, de noradrenerge neuronen in de locus coeruleus en subcoeruleus, en vermoedelijk ook in bepaalde delen van de nucleus reticularis inferior, en in verscheidene peptiderge cellen in de hersenstam Deze banen spelen waarschijnlijk een belangrijke rol, samen met de substance P vezels afkomstig uit de spinale ganglia, in de regulatie van de pijnwaarneming

NAWOORD

Gaarne wil ik mijn oprechte dank uitspreken aan allen die mij in de periode van 1 september 1980 tot 1 september 1984, en gedurende de overvolle maanden daarna rechtstreeks geholpen hebben met de werkzaamheden die moesten leiden tot de totstandkoming van dit proefschrift Mijn bijzondere dank gaat uit naar hen, die zich in de vele situaties waaraan de druk van de tijd een gespannen en soms onaangenaam karakter verleende, loyaal toonden, door niet alleen de van hen verlangde taken met zorg te vervullen, maar ook de vaak rigide arbeidswetten met soepelheid te betrachten, de weinige sluipwegen in het formeel-organisatorische universitaire net optimaal te benutten, werkzaamheden te verrichten die buiten hun taakomschrijving vielen of hen van hogerhand geheel ontzegd waren, ja, door zo nodig zelfs een aanslag te doen op de eigen vrije tijd Het lijdt geen twijfel dat deze mensen op cruciale momenten een pak van mijn hart hebben genomen

Op een ander vlak wil ik mijn intense dank uitspreken aan al diegenen, die met hun opgeruimde blijmoedigheid, hun open oor en begrip, hun stimulerende opmerkingen, en hun positieve kijk op de medemens, mij geholpen hebben vrijwel onafgebroken de vreugde in het bestaan te blijven ervaren, en de toekomst met vertrouwen tegemoet te zien Het spreekt vanzelf dat deze steun een conditio sine qua non is geweest

In dezelfde sfeer ligt mijn dankbaarheid ten aanzien van de muze, die naast die der wetenschap zo'n grote rol in mijn leven speelt. die der toonkunst De vruchten van haar inspirerende kracht gedurende de laatste vijf eeuwen, gedragen door talrijke uitnemende componisten, maar ook haar dagelijkse werkzaamheid in mij en om mij heen hebben mij zeer verrijkt, en mede de energie geleverd voor de arbeid aan dit proefschrift. Gelet op de nauwe verbintenis tussen de kunsten en wetenschappen van oudsher en in vele esoterische stromingen, lijkt een wederzijdse positieve beinvloeding ook zeer waarschijnlijk. Zo geldt mijn dankbaarheid in dit kader ook hen, die mij in staat hebben gesteld samen met hen de muziek in zovele aspecten te (leren) beoefenen en te genieten.

Tot slot enkele namen. De analytische ondersteuning was in de handen van Roelie de Boer, die zich onmisbaar maakte door de ontelbare duistere uren achter het fluorescentiemicroscoop en het telwerk dat aan de basis lag van de resultaten van het onderdeel collateralen (Hoofdstuk 6); Jos Dederen, die de veilige en betrouwbare leidsman was door de experimenten met de hoogste activiteit, nl de radioactiviteit van het getritieerd leucine (Hoofdstuk 7), waarvoor hij, samen met Rene van Rheden, het histologische en fotografische werk geheel op zich nam, Henk Joosten, die verscheidene immunohistochemische modelseries van de varaan vervaardigde (Hoofdstukken 3,4 en 5), Annelies Pellegrino, die de lijntekeningen behorende bij de immunohistochemische hoofdstukken met veel geduld en grote nauwkeurigheid heeft berasterd; Henk van de Locht, die met raad en daad de aanschaf en het optimale gebruik van mijn personal computer en de uitgebreide software heeft begeleid

Het secretariaat Wanda de Haan, die, na de manuscripten voor de eerste vier hoofdstukken op conventionele wijze uitgetypt te hebben, de moed en het geduld had om ten behoeve van dit proefschrift zich voor slechts korte tijd in te werken in het Wordstar programma op mijn machine

De tekenaars Joep de Bekker, Chris van Huyzen, Wil Maas en Nol van Uden, die onder vaak extreme tijdsdruk hun fraaie bijdragen wisten te realiseren.

De dierenverzorgers Hennie Eikholt, Gerrie Grutters, Hendrik-Jan Janssen en Piet Spaan, die het onvermijdelijke lijden en het noodlottig einde van de proefdieren onder de meest gunstige omstandigheden in het Centraal Dierenlaboratorium met hun zorg omkleedden

De fotografen Cor de Bruyn, Ton van Eupen, Flip Fransen, Fred Hoogendoorn en Han Luikens, die met grote vakkundigheid het zeer moeilijke fotowerk optimaal hebben verzorgd, en mij, tijdens de vele uren die ik met hen in de DoKa doorbracht, hebben ingewijd in de fijne kneepjes van het handwerk afdrukproces

De buitenwacht· voor verschillende onderdelen van mijn studie waren de kontakten met wetenschappers op andere instituten zeer waardevol, en hun gastvrijheid was hartverwarmend, Heleen Barbas (Anatomie, Vrije Universiteit, Amsterdam) heeft mij bij het samenstellen van de atlas (Hoofdstuk 1) bijzonder geholpen met haar experimenteel verworven kennis omtrent de lokalisatie van verschillende hersenzenuwkernen; Gert Holstege (Anatomie, Erasmus Universiteit, Rotterdam) heeft ons de sleutel tot de autoradiografische techniek helpen zoeken (Hoofdstuk 7), Margriet Huisman en Koos Keyzer (Anatomie, Erasmus Universiteit, Rotterdam), beiden wetenschappelijk opgegroeid naast de wieg van de multiple retrograde fluorescerende tra-

cer techniek, hebben mij op verschillende punten met raad en daad bijgestaan met het verkrijgen en optimaal verwerken van gegevens met deze techniek; Harry Steinbusch, vriend en dorpsgenoot, heeft mij vanaf mijn eerste stappen op het immunohistochemische vlak deskundig en kritisch begeleid, stond garant voor desnoods grote hoeveelheden anti-serotonine serum, en was een inspirerende reisgezel op onze gemeenschappelijke congresreizen naar Zweden (Lund), Spanje (Torremolinos) en Duitsland (Hamburg).

Tot slot "hors catégorie": mijn naaste medepromovendi en vrienden Loek Leenen en Peter van Mier, met wie ik welhaast alle lief en leed heb gedeeld. De brede basis van onze betrokkenheid op elkaar gaat verder dan alleen het bezit van drie identieke personal computersystemen, al heeft de moderne technologie toch zeker daartoe bijgedragen. Met zijn ervaring op het gebied van getallen-verwerking, statistiek, en de omgang met computersystemen vormde Loek niet alleen de meest ideale vraagbaak voor het bewerken van de gegevens van de dubbellabelings experimenten (Hoofdstuk 6); hij leverde ook de voor dit onderdeel benodigde programmatuur. Ook Peters bereidwillig toegestoken helpende hand, en zijn waardevolle adviezen op het visueel-esthetische vlak zijn op vele plaatsen in dit proefschrift terug te vinden.

CURRICULUM VITAE

Jan Wolters werd geboren op 16 januari 1953 te Baarle-Nassau. Hij doorliep het Gymnasium β aan het Onze Lieve Vrouwelyceum te Breda waar hij in 1971 het einddiploma behaalde. Vervolgens begon hij zijn opleiding tot arts aan de Katholieke Universiteit te Nijmegen, waar hij in 1974 het kanditaatsexamen en in 1977 het doktoraal examen aflegde. Gedurende deze periode volgde hij tevens de colleges muziekwetenschap, en verscheidene cursussen psychologie, fonetiek, en algemene taalwetenschap. Als student-assistent was hij regelmatig werkzaam binnen de vakgroepen Anatomie en Embryologie en Sociale Geneeskunde. In 1978 verrichtte hij op de afdeling Neurofysiologie van de KU een origineel onderzoek naar veranderingen in de spiertonus bij het luisteren naar muziek. Sinds 1978 is hij als free-lance muziekmedewerker verbonden aan de Gelderlander Pers. In 1980 schreef hij een sociaal geneeskundige scriptie over aspekten van geluidhinder bij geringe geluidsintensiteit. Na het behalen van het artsexamen in 1980 was hij van 1 september 1980 tot 1 september 1984 verbonden aan de vakgroep Anatomie en Embryologie, waar dit proefschrift werd bewerkt.

STELLINGEN

behorende bij het proefschrift "On the Anatomy of Descending Pathways from the Brain Stem to the Spinal Cord in a Lizard, *Varanus exanthematicus*", door Jan Wolters.

1 De gebruikelijke neiging van neuroanatomen om aan gelaagde strukturen het begrip 'hogere ordening' te verbinden dient te worden toegeschreven aan het onvermogen om in ongelaagde strukturen een duidelijke morfologische ordening te ontdekken

2 Het lijkt waarschijnlijk dat de zogenoemde derde komponent van het motorisch systeem een belangrijke rol speelt in de emotionele kleuring van het gedrag

H G J M Kuypers (1982) A new look at the organization of the motor system In. H G.J.M Kuypers and G F. Martin (eds)· *"Descending Pathways to the Spinal Cord"*, Amsterdam· Elsevier, *Prog.Brain Res.* Vol. 57, pp 381-403

3. De belangrijke bijdrage aan de innervatie van de achterhoorn door projecties die ontspringen in de raphe kernen en de locus coeruleus zou een verklaring kunnen vormen voor de ruime mate van pijnonderdrukking onder omstandigheden welke een hoge motorische aktiviteit vereisen, zoals 'fight' en 'flight' reakties.

4. Binnen de universitaire organisatie zal decentralisatie en schaalverkleining op het gebied van dienstverlening, van facultair- naar vakgroepniveau, in veel gevallen leiden tot kwaliteitsverbetering en een grotere mate van efficientie.

5 Preventieve gezondheidszorg is, met name bij de opsporing en behandeling van symptoomloze risicofaktoren, in de eerste plaats gebaat bij een rigoureuze systematiek.

F.J A Huygen, afscheidscollege "Levensloop en Ziekte", 5 okt. 1985.

6. Niet de groep patienten die zich regelmatig bij de huisarts meldt, maar juist dat deel van de 'patienten'populatie dat zich nimmer laat zien vormt de grootste risicogroep in het verwerven van preventief te benaderen somatische en psychosociale ziektebeelden

7. Mits vroegtijdig onderkend zijn veel conversie-reacties, vooral wanneer deze ontstaan zijn als reactie op zware stress, met redelijk tot goed resultaat te behandelen.

F.C. Moene en A. Groenenboom (1985) Ned.Tijdschr.Geneeskd 129:904-907.

8. Onder zangeressen en zangers komt nutteloos en onverantwoord antibioticagebruik in ruime mate voor.

9. Het bijzondere van de zangkunst binnen het geheel van muzikale uitingen is, dat zij spraak en muziek, functies die gereguleerd worden door een verschillend neuraal substraat, kombineert.

10. De halsspieren zijn, in tegenstelling tot andere spiergroepen, geneigd zich te ontspannen bij het luisteren naar muziek.

J.G. Wolters (1979) Keuzestage-projekt Neurofysiologie.

11. Uit de getallensymboliek in grote delen van het oeuvre van Johann Sebastian Bach blijkt dat de componist reeds op 22-jarige leeftijd zijn preciese sterfdatum heeft gekend.

K. van Houten en M. Kasbergen (1985) in: "Bach en het Getal", Zutphen: de Walburg Pers.

12. Het lijkt verstandig reeds tijdens de opvoeding te anticiperen op de problemen die geluidhinder kan veroorzaken, door het kind enerzijds te trainen in een zekere mate van tolerantie voor geluiden die anderen produceren, en het anderzijds een besef bij te brengen van de mate waarin geluidsproduktie zonder schadelijk te zijn voor anderen, toegestaan en zelfs aan te raden is.

13. De nadruk die de meeste politieke partijen en vakbonden heden ten dage leggen op bestrijding van de werkeloosheid heeft een negatieve invloed op de algemeen maatschappelijke acceptatie van de onvermijdelijk toenemende vrije tijd, en op de ontwikkeling van een levensstijl die meer aandacht besteedt aan intrinsieke persoonlijke drijfveren, aan een sociaal bewustzijn, en een veelzijdige creativiteit.

14. Modemverbindingen stellen vooralsnog hoge eisen aan de deskundigheid en het geduld van de gebruikers.

15. Niet de feiten overtuigen, maar feiten die passen in een geloof.

P. Vroon (1976) in: "Weg met de Psychologie", Baarn: Ambo.

16. Een selecte steekproef op het vaderlandse autosnelwegennet heeft uitgewezen, dat personenauto's van Nederlandse makelij, variërend in leeftijd van 8 tot 25 jaar, in betrouwbaarheid, degelijkheid en comfort niet onder hoeven te doen voor veel recentere modellen.